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FACTORS AFFECTING THE GERMINATION OF SUGAR-BEET AND OTHER SEEDS, WITH SPECIAL REFERENCE TO THE TOXIC EFFECTS OF AMMONIA[1]

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INTRODUCTION

The presence in sugar-beet seed balls of water-soluble substances that produce a toxic effect on germinating seed has been shown in a previous publication (*25*).[3] In the present report the nature of the toxic action is analyzed and the effects of osmotic pressure and of hydrogen-ion concentration are evaluated. Evidence is presented that free ammonia, released during germination from nitrogenous compounds in the water extracts of the seed balls, is an important and significant factor in germination inhibition and that this free ammonia produces toxic effects when sugar-beet seed is germinated in the presence of the seed-ball extracts.

REVIEW OF LITERATURE

Extensive work has been reported on the factors affecting the germination of seeds of fleshy fruits when placed on a substratum moistened with the fruit juice. According to Reinhard (*18*), Molisch was the first to express the opinion that the retarded germination was caused by inhibiting substances. The opinion was later supported by Oppenheimer (*14*, *15*), Fukaki (*6*), and Reinhard (*18*). Oppenheimer suggested that the inhibitory substances are colloidal and that they act as "negative catalysts" influencing respiratory processes. Köckemann (*9*) described inhibitory substances, found in various fleshy fruits, that were soluble in water and in ether and were heat-resistant but were destroyed by peroxide and alkali. Reinhard (*18*), working with tomato juice, found inhibiting substances that were water-soluble and heat-resistant but were not destroyed by neutralization.

Osmotic pressure of fruit juices has been considered by some workers a factor in the inhibition of germination. Oppenheimer (*14*, *15*) and Fukaki (*6*) stated that the inhibitory action of substances in tomatoes is something in addition to osmotic effect because boiling the juice increased the osmotic pressure while it reduced the inhibitory action. Recently, Litvinov (*12*) stated that the essential cause of the inhibiting effects of fruit juices is osmotic pressure and that only slight inhibition is traceable to specific organic substances. This conclusion is similar

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[3] Italic numbers in parentheses refer to Literature Cited, p. 711.

to that of Lavialle (*10*), who stated that seeds in all fleshy fruits remain dormant because of the osmotic pressure of their juices, which may vary from 5 atmospheres in tomatoes to 20 atmospheres in grapes.

Other observations pertinent to the studies here reported may be noted. Axentjev (*2*) reported that the inhibiting action of seed extracts is not dependent upon fluorescence. He stated that the action of *Phacelia* seed extract was not specific but that it inhibited the germination of some seeds and stimulated that of others. Mosheov (*13*) reported the presence in wheat of germination-inhibiting substances that were heat-labile and were most effective in light. Lehmann (*11*) found substances having similar properties in the hulls of buckwheat. On the basis of tests with the seeds of *Vaccaria pyramidata*, Borriss (*3*) concluded that the increased germination of seeds planted in soil was not due to any stimulative effect of the soil but rather to the removal of inhibitory substances from the seed by adsorption. Shuck (*20*, *21*) reported inhibitory substances, produced by germinating lettuce seed, that were water-soluble and probably a metabolic byproduct. Borthwick and Robbins (*4*) pointed out the possibility that there are deleterious products of metabolism in the endosperm or embryo of lettuce seed, arising and possibly accumulating only at higher temperatures, and that, when other parts are removed, the naked lettuce embryo germinates at these higher temperatures. Thompson (*22*) and Thompson and Kosar (*23*) found soaking and drying, both at low temperatures, and soaking in certain chemical solutions to be effective in breaking the dormancy of lettuce seeds.

MATERIALS AND METHODS

The sugar-beet (*Beta vulgaris* L.) seed balls used in these studies as sources of extracts were principally of two varieties, 68 and U. S. 12. Only one seed lot of variety 68 was available. Previous studies had shown this seed lot to be a heavy carrier of substances that resulted in a toxic effect on germination. The following four lots of seed balls of U. S. 12 were used; these were grown under widely different conditions and varied greatly in chemical composition.

U. S. 12–A.—Normal soil, Hurricane, Utah, 1936 crop. This seed lot was very low in material toxic to germinating seeds.

U. S. 12–B.—Alkali soil, St. George, Utah, 1938 crop. Grown on soil showing considerable white alkali but with fairly good plant survival.

U. S. 12–C.—Extreme alkali soil, St. George, Utah, 1938 crop. Grown on the same field as U. S. 12–B, but in areas where, owing to high alkali content, only a few plants survived and produced seed.

U. S. 12–D.—Normal soil, Moab, Utah, 1938 crop. Owing to insect damage or some other environmental influence, most plants producing this lot of seed reverted to vegetative type of growth during the period when seed normally sets and matures. Viability of the seed was very poor.

Two samples of lettuce seed of the Grand Rapids variety were obtained from T. W. Whitaker.[4] Other kinds of seed such as tomato, radish, cantaloup, cucumber, etc., were obtained from a commercial seed store in Salt Lake City, Utah. These are listed in the various tables.

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The relative toxicity or germination-inhibiting properties of the extracts or solutions tested were measured by their ability to decrease germination percentages under standardized conditions.

A distinction is sometimes necessary between toxicity and inhibition of germination because certain factors, such as high osmotic pressure, may inhibit germination without resulting in a toxic effect.

The term "toxic substances" as used throughout this report is not limited in meaning to actual substances that are toxic to germinating seeds or plants, but also refers to substances that may not be toxic themselves but from which the toxic agent is produced during the course of seed germination. For example, dilute solutions of asparagin allowed germination to proceed normally for about 48 to 72 hours before sufficient ammonia was released to stop further growth and actually kill the plants.

The seed-ball extracts were prepared by soaking quantities of air-dry seed balls in five times their weight of distilled water for 20 hours, and filtering. Toxicity was determined by germinating naked sugar-beet seeds on thin cotton substrata in 3½-inch Petri dishes moistened with 9 ml. of the solution being tested. Variety 68 was the source of all naked sugar-beet seeds used in the tests.

Osmotic pressure measurements were made by the cryoscopic method, using an air-jacketed sample tube and Beckmann differential thermometer. A glass-electrode pH meter was used in making all pH measurements. Where the pH of extracts or solutions from germination dishes was determined, the sample was obtained by folding the cotton substratum on which the seeds were placed and pressing out the excess solution.

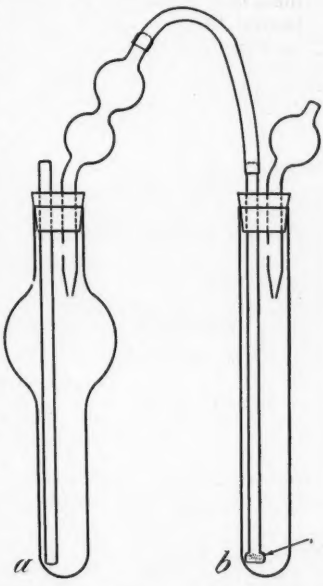

FIGURE 1.—One unit of ammonia aspiration apparatus. *a*, Aspiration tube; bulb blown for larger samples and to help reduce foaming. *b*, Absorption tube; sintered glass disk in delivery tube and two drops of *n*-butyl alcohol used in solution to insure complete absorption of ammonia.

The nitrogen fractions (free ammonia,[5] combined ammonium salts, and amide nitrogen) were determined by aspirating into boric acid and measuring the conductivity of the solution as outlined by Hendricks et al. (*7*). The aspiration and absorption apparatus was patterned somewhat after that outlined by Sessions and Shive (*19*). One unit is shown in figure 1. A bank of 12 units with accessory equipment is shown in figure 2.

[5] The term "free ammonia" as used in the present report refers to that ammonia which can be aspirated from a solution without addition of alkaline reagent. Combined ammonium nitrogen refers to the additional ammonia released from its salts by a suitable alkaline reagent. This distinction was made in the present instance even though "free ammonia" has sometimes been used in the literature to indicate ammonia derived from the decompositions of all ammonium salts. The authors consider the latter usage clearly a misnomer.

The *n*-butyl alcohol and sintered glass disks in delivery tubes insured complete absorption, while the warm-water bath and reduced pressure very greatly shortened the time necessary for complete removal of ammonia from the sample. No appreciable phosphate was present in the samples; therefore, magnesium oxide was used as the alkaline reagent. This was done to keep the volume of solution to a minimum for amide nitrogen determination.

The procedure was as follows: 15 ml. of 0.4 molar boric acid solution and 2 drops of *n*-butyl alcohol were added to each absorption tube, and the stopper was pressed firmly in place; 10 ml. of the solution to be analyzed and a drop of mineral oil (to prevent foaming) were added to the sample tubes, the rubber tubes were connected, and aspiration was started. All analyses were run in duplicate or triplicate. Duplicates usually agreed within 0.02 mg. of nitrogen. After

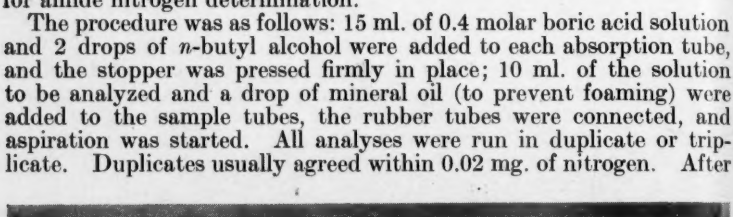

FIGURE 2.—Twelve units of the ammonia aspiration apparatus with accessory equipment. *a*, Suction trap with Bunsen valve. *b*, Hot-water bath for aspiration tubes made from 3-inch galvanized-iron pipe, slightly flattened on sides; vented outlet bent down inside to remove water from the bottom of bath. *c*, Air-scrubber bottles; stopcock for adjusting air intake when operating under reduced pressure. *d*, Hot-water heating coil. Small constant-level feed tank not shown. Note depth of fine bubbles in absorption tubes.

aspiration the tubes were disconnected, starting from the end opposite to that at which suction was applied. Suction was decreased gradually as the tubes were disconnected. The contents of the absorption tube was made up to 100 ml. thoroughly mixed, and the conductivity determined. The nitrogen value was read from graphs prepared from conductivity values obtained by adding a known quantity of ammonia to 15 ml. of boric acid and sufficient distilled water to make 100 ml. The plotted curves were nearly straight lines. Combined ammonium nitrogen was determined by adding a small excess of dry magnesium oxide (MgO) to the sample tube and aspirating into fresh boric acid. The acid hydrolysis with 10-percent sulfuric acid, described by Orcutt and Wilson (*16*), was employed for amide nitrogen. After hydrolysis the samples were rinsed back into the original sample tubes and a predetermined amount of strong sodium hydroxide (NaOH), sufficient to nearly neutralize the acid, was added down the side of the tube by means of a pipette. A drop of mineral oil, a drop of phenolphthalein indicator, and an excess of magnesium oxide was added, the flask was immediately closed, and the aspiration was started. Residual nitrogen was determined on the same sample by the Gunning method for organic and ammoniacal nitrogen, with 0.04 N acid and base.

EXPERIMENTAL WORK AND RESULTS

SOME CHEMICAL AND PHYSICAL PROPERTIES OF SUGAR-BEET SEED-BALL EXTRACTS

Water-soluble extracts from sugar-beet seed balls of different varieties and from different seed lots of the same variety varied rather widely in color. The color of fresh extracts usually ranged from amber to dark brown.

All extracts became darker on exposure to the air and darkened rapidly when alkali was added. Normal color was restored upon the addition of excess acid. The extracts turned very dark when ferric chloride was added to them, and a white precipitate was formed with stannous chloride, gelatin, or lead acetate. A voluminous precipitate of gums and pectins was obtained when 95-percent alcohol was added to the concentrated extract. The extracts readily reduced potassium permanganate. Many of the above tests indicated the presence of tannins and gums. Germination tests, however, on pure solutions of tannic acid, gum arabic, etc., indicated that the amounts present could not be responsible for any appreciable degree of toxicity.

Determinations of the heavy metals by means of the dropping mercury electrode showed copper, lead, and zinc to be too low to cause injury.

The fact that the toxicity of the water-soluble extract is due to heat-stable, colloidal, organic matter was indicated by the following tests: The toxicity of the water-soluble extract was reduced when the extract was filtered through one-half of an inch of diatomaceous earth, and it was still further reduced when carbon black was used with the diatomaceous earth. The toxic nature of the extract was not affected by high temperature. Table 1 shows a comparison of the toxic effect of untreated seed-ball extract with that of an extract which had been steamed for 30 minutes at 20 pounds' pressure. When the extract was evaporated to dryness and the residue redissolved in distilled water, little or no change in the effect on germinating seeds was evident. Some of the substances that retarded germination remained in the water-soluble ash, although the effect was not characterized by darkening and killing of the sprouts, as in tests with the normal extract. That retardation in this case could be accounted for by osmotic pressure alone was shown by subsequent tests.

TABLE 1.—*Toxicity of heated and normal extracts from sugar-beet seed balls as indicated by their power to inhibit the germination of naked sugar-beet seeds*

[Average of 4 varieties]

Treatment of extract	Naked seeds germinated after—		
	19 hours	42 hours	90 hours
	Percent	*Percent*	*Percent*
Steamed 30 minutes at 20 pounds' pressure	17.67	39.83	60.70
Untreated	18.33	43.50	59.60

The solubility of the toxic materials of the seed-ball extracts in various solvents was determined by evaporating a known volume of original extract to near dryness, extracting with a solvent, removing the solvent by successive evaporations with water, redissolving the

residue in distilled water to the original volume, and germinating seeds on the various fractions. The toxic materials were found to be soluble in water, or in 80-percent alcohol. They were slightly soluble in absolute alcohol and practically insoluble in propyl alcohol, ether, acetone, or toluene.

Some of the chemical and physical properties of typical sugar-beet seed-ball extracts are shown in table 2. The close correlation between ash, specific conductance, and osmotic pressure indicates that while the total amount of inorganic salts in the extracts varies the relative proportions of the constituent salts remain nearly the same. None of the indices of inorganic salts correlates well with toxicity.

TABLE 2.—*Some properties of sugar-beet seed-ball extracts as related to toxicity*

Source of extract	Osmotic pressure	Total solids	Ash	Specific [1] conductance $\times 10^{-5}$	Total nitrogen	Surface tension [2] per centimeter	Naked seeds germinated after 121 hours
	Atmospheres	*Percent*	*Percent*		*Percent*	*Dynes*	*Percent*
Variety 68 (normal extract)	8.48	1.97	1.087	120.73	0.0645	51.4	3
Variety 68 (filtered through kieselguhr and carbon black)	6.08	1.73	.992	119.54	.0582	.....	12
U. S. 12-A	6.08	1.35	.737	83.06	.0533	.....	50
U. S. 12-B	9.62	2.28	1.201	136.52	.0672	47.4	31
U. S. 12-C	12.27	3.15	1.556	171.91	.1329	49.6	22
U. S. 12-D	4.28	1.37	.766	62.10	.0884	.....	21

[1] Reciprocal ohms per centimeter $\times 10^{-5}$; 5 ml. diluted to 100 ml. for conductivity measurement.
[2] Surface tension of distilled water at 25° C.=71.97 d. per centimeter.

OSMOTIC PRESSURE

Two different types of solutes, sucrose and sodium chloride, were used to determine the tolerance of naked sugar-beet seeds to osmotic pressure. This was done to check any effects that might be due to factors other than osmotic pressure. Table 3 shows that sugar-beet seeds are tolerant to fairly high osmotic pressure and that the two solutions used gave fairly similar results.

TABLE 3.—*Influence of osmotic pressure on the germination of naked sugar-beet seeds*

Solute used	Osmotic pressure	Naked seeds germinated after—			
		25 hours	49 hours	73 hours	142 hours
	Atmospheres	*Percent*	*Percent*	*Percent*	*Percent*
Water (check)	0	64	88	99	99
Sucrose	2.12	67	85	96	99
	4.29	67	89	93	97
	6.12	55	69	79	85
	8.40	46	67	77	82
	12.55	24	40	62	69
	18.53	4	7	14	28
	23.67	0	0	3	13
Sodium chloride	2.00	75	88	94	96
	3.94	60	74	85	87
	5.78	46	69	78	84
	7.73	38	66	67	74
	11.28	15	35	50	57
	15.55	8	21	34	35
	19.54	0	4	12	13

Several other solutes were used in concentrations having osmotic pressure values similar to that of variety 68 sugar-beet seed-ball extract. The data in table 4 show that these isotonic chemical solutions varied in their influence on seed germination. Differences in germination up to 70 percent were observed on solutions having similar osmotic pressure. Urea, for example, was outstanding in toxicity. The tips of the radicles from the seeds that germinated in the presence of urea were similar in appearance to those of radicles from seeds germinated on sugar-beet seed-ball extracts.

It is evident from the data that important toxic effects on germinating seed cannot be explained on the basis of osmotic pressure.

TABLE 4.—*Influence of sugar-beet seed-ball extracts and of chemical solutions isotonic with certain seed-ball extracts on the germination of naked sugar-beet seeds*

Extract or solution used to moisten substratum	Osmotic pressure	Naked seeds germinated after—		
		22 hours	46 hours	121 hours
Sugar-beet variety:	*Atmospheres*	*Percent*	*Percent*	*Percent*
68 (extract)	8.48	0	3	3
68 (extract diluted with 2 parts water)	3.36	9	9	25
68 (extract filtered through kieselguhr and carbon black)	6.08	0	4	12
Urea	8.25	1	2	3
Ammonium sulfate	8.48	13	33	39
Sodium nitrate	8.57	13	29	39
Sodium chloride	8.48	17	45	57
Sucrose	8.37	25	63	73
Water (check)	0	54	92	94

TOXICITY OF AMMONIA

Release of Ammonia During Germination

The known instability of urea and a faint odor from the urea, similar to that from seed-ball extracts, suggested that ammonia might be released during germination.

Germination tests were conducted with naked sugar-beet seeds on seed-ball extracts and chemical solutions hermetically sealed in glass dishes. After 71 hours 2 liters of ammonia-free air was passed through the dishes and Nessler's solution. The results of this test showed that ammonia was liberated from all the seed-ball extracts and solutions containing nitrogen and that there was an increase in alkalinity during germination (table 5). A comparison of the effect of the dilute ammonia and urea solutions (table 5) shows that the concentration of ammonia was greatest at the beginning of the test in the dishes containing the ammonia solution; germination was completely inhibited, and alkalinity was high but decreased during the test. The dishes containing urea solutions started with a comparatively low pH and low initial concentration of ammonia, which allowed some germination to start. During the test, however, the ammonia and alkalinity both increased to the point where more ammonia was in equilibrium with the air in the dishes containing urea than in those containing the ammonia solution. The behavior of asparagin solutions in this test was also interesting. Evidently very little ammonia was released until after about 2 days, when some apparently normal germination had taken place. Soon after this, however, the seedlings turned brown and no further germination occurred. This delayed action was evident in all subsequent tests with asparagin.

TABLE 5.—*Free ammonia content of air drawn through vessels containing naked sugar-beet seeds germinating on various extracts and solutions*

Extract or solution	Osmotic pressure	pH of fresh solution	Results after 71 hours		
			pH	Naked seeds germinated	Nitrogen[1]
Sugar-beet variety:	*Atmospheres*			*Percent*	*Mg.*
68 (extract)	8.48	6.27	8.20	9	0.027
U. S. 12-A (extract)	6.08	5.50	7.65	29	.009
U. S. 12-B (extract)	9.62	5.20	7.80	20	.010
U. S. 12-C (extract)	12.27	4.75	7.54	16	.010
U. S. 12-D (extract)	4.28	5.50	8.16	21	.015
Ammonia (0.4 mg. N per ml.)		10.50	8.16	0	.050
Urea (1.9 percent)	8.25	7.00	8.55	3	.080
Urea+1 percent NaCl[2]	8.30	5.13	8.06	15	.040
Asparagin (4.2 percent)			8.00	31	.060
Asparagin+1 percent NaCl[2]	8.30	4.33	8.10	40	.030
Sodium nitrate	8.57	5.75	7.72	32	.009
Ammonium sulfate	8.48	5.20	6.48	35	.005
Water (check)			6.20	96	0

[1] As ammonia in 2 liters of air.
[2] Contained approximately 1 mg. of nitrogen per milliliter.

Further evidence on the relation of the rapidity of ammonia release from nitrogenous compounds to toxicity was obtained in an experiment with seed-ball extracts. In this test the ammonia from the most readily broken down nitrogen compounds was removed from the seed-ball extract, and the toxicity of the modified extract was then compared with a portion of the same extract from which practically no ammonia had been removed. Two hundred milliliters of heated seed-ball extract was divided and 25 ml. of an extract from germinating seeds was added to one of the 100-ml. portions (*A*). An equal amount of heated extract from germinating seeds was added to the

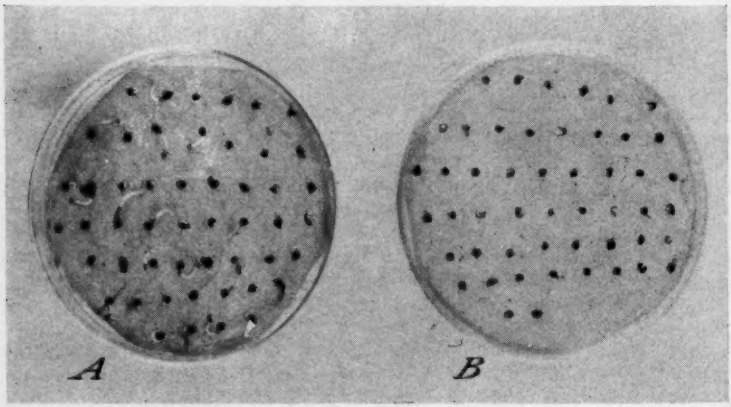

FIGURE 3.—Relation of the nitrogen content of seed-ball extracts to delayed toxicity: *A*, Seed-ball extract from which 0.272 mg. of nitrogen as ammonia per milliliter was removed prior to testing toxicity; *B*, seed-ball extract from which practically no nitrogen had been removed prior to testing toxicity. Note almost complete inhibition of germination in *B* and delayed toxicity in *A*; darkening of sprouts in *A* is typical of ammonia injury.

other portion (*B*) of seed-ball extract, and both mixtures were incubated for 4 days. Both samples were then aspirated; 0.272 mg. of nitrogen as ammonia per milliliter of solution was removed from *A*, while only 0.002 mg. was removed from *B*. Both samples were then heated to stop further action, and their toxicity to germinating seeds compared. Although the final pH of both lots after 90 hours of germination was practically the same and sprouts from both samples were ultimately killed, germination counts showed that the removal of more ammonia from *A* resulted in increased germination (fig. 3, *A* and *B*). Forty-eight percent of the seed on the seed-ball extract from which very little ammonia had been removed germinated in 90 hours, while 81 percent of the seed on the seed-ball extract from which the larger amount of ammonia had been removed germinated.

Toxic Effect of Ammonia on Sugar-Beet Seeds and Seedlings

To obtain further information on the effect of ammonia, tests were conducted to determine the tolerance of seedlings and older sugar-beet plants to ammonia solutions and sugar-beet seed-ball extracts. Three-week and two-month-old plants were supported through corks and their roots submerged in seed-ball extracts and in an ammonia solution containing 0.4 mg. of nitrogen as ammonia per milliliter. All of the smaller plants collapsed within 4 hours. The roots of the larger plants turned black, and the leaves began to dry in 18 hours. Water checks started at the same time were still living at the end of 2 months.

In another test small seedlings were placed in water culture and the vials put in a larger container. Enough of the above-mentioned ammonia solution was placed in the large container to cover the bottom to a depth of one-quarter of an inch, and the large container was then sealed. Although the small seedlings were not in contact with the ammonia solution, within 2 hours sufficient ammonia had accumulated within the aerial parts to cause their collapse. Subsequent tests, reported in table 6, showed that ammonia concentrations above 0.1 mg. of nitrogen per milliliter decreased germination of naked sugar-beet seeds. Further tests (see fig. 4) indicated that much lower concentrations of ammonia, if effectively maintained, would inhibit germination.

Table 6.—*Influence of several different concentrations of free ammonia on germination of naked sugar-beet seeds*

Nitrogen[1] (milligrams)	pH of fresh solution	pH of solution after germination	Naked seeds germinated after—		
			22 hours	65 hours	120 hours
			Percent	*Percent*	*Percent*
Water (check)	5.57	6.20	53	95	95
0.025	8.50	6.51	47	96	96
.050	9.35	7.01	39	93	93
.100	9.90	7.51	21	69	74
.200	10.20	8.12	12	56	57
.300	10.30	8.30	7	12	14
.400	10.50	8.40	0	0	0
.800	10.72	8.53	0	0	0

[1] As ammonia per milliliter of solution.

Nitrogen Analysis of Sugar-Beet Seed-Ball Extracts and Chemical Solutions Before and After Germination

To determine whether toxic amounts of ammonia were released from certain chemical solutions and seed-ball extracts by germinating seed, nitrogen analyses were made on seed-ball extracts and chemical solutions immediately after their preparation and also after their recovery from the cotton substratum on which naked sugar-beet seeds had been germinated for 5 days. The results of these analyses are shown in table 7. Free ammonia was liberated from the nitrogen compounds of all the extracts and synthetic solutions. The presence of the liberated ammonia is reflected in the rise in pH. The pH values after germination (table 7) did not rise so high during germination as in several other instances where germination was conducted in smaller dishes and on thinner substrata. The significant data in table 7 are the increases in the amount of free ammonia in the substrata after seed germination. The amounts in most instances are more than sufficient to cause inhibition of germination, especially since the ammonia was released during germination and tended to increase as germination proceeded rather than to be decreased by loss or neutralization, as was evident in tests where ammonia solutions were used. The amounts of combined ammonium ion serve to indicate the relative buffering capacities of the solutions or extracts. No significance was attached to the small changes in amide nitrogen. Residual and total nitrogen determinations were merely checks on technique.

Table 7.—*Analyses of the nitrogenous constituents of sugar-beet seed-ball extracts and solutions of urea and asparagin*

[Analyses of fresh solutions and of solutions recovered from substrata on which seeds had been germinated]

Source of extract or synthetic solution	Fresh solution						Solution after germination (5 days)					
	pH	Nitrogen [1] as—					pH	Nitrogen [1] as—				
		Free NH_3	Combined NH_4^+	Amide N	Residual N	Total N		Free NH_3	Combined NH_4^+	Amide N	Residual N	Total N
Sugar-beet variety:		*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*		*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
68	6.27	0	0.081	0.199	0.398	0.678	7.70	0.315	0.044	0.159	0.157	0.675
U. S. 12-A	5.50	0	.049	.066	.368	.483	7.58	.228	.067	.034	.201	.530
U. S. 12-C	4.75	0	.100	.132	1.097	1.329	7.64	.364	.216	.187	.575	1.342
U. S. 12-D	5.50	0	.073	.163	.666	.902	7.55	.370	.020	.066	.361	.817
Urea+1 percent NaCl	5.13	0	.001	.198	.799	.998	7.58	.146	.034	.325	.534	1.039
Asparagin +1 percent NaCl	4.33	0	.000	.386	.437	.823	7.58	.707	.085	.012	.102	.906

[1] Measured as milligrams per milliliter of solution.

Ammonia Released by Enzymatic Hydrolysis

Further studies were conducted to determine the source of the hydrolytic agent responsible for the release of free ammonia from seed-ball extracts and certain chemical solutions containing organic nitrogen. Two sets of Petri dishes were prepared for germination tests. The substrata of one set were moistened with sugar-beet seed-ball extract and those of the other with 0.25-percent urea solution. The dishes were covered and steamed at 20 pounds' pressure for 30

minutes and were then cooled, and naked sugar-beet seeds that had been shaken with toluene were placed in one-half of each lot of dishes. All planting was done under a General Electric germicidal lamp (2,537 A. U. radiation) in a closed inoculation chamber. After 93 hours the liquid was recovered from the dishes and immediately analyzed. The result of the analysis (table 8) indicates that the seed carries a hydrolytic agent that is probably enzymatic in character.

Tests were run in which germinating seeds were ground and extracted with water. This extract was added to sugar-beet seed-ball extract and chemical solutions, and analyzed immediately after mixing and after periods of incubation at 30° C. One flask containing seed-ball extract and the above-mentioned extract from germinating seed was heated at 20 pounds' pressure for 30 minutes. The analyses are presented in table 9. It is evident from the data in table 9 that an extract prepared from germinating beet seed has hydrolytic properties similar to those of intact germinating seeds and that this hydrolytic agent is heat-labile.

TABLE 8.—*Nitrogen released by germinating sugar-beet seeds under sterile conditions*

[Seeds were washed in toluene]

Treatment	pH	Nitrogen as free ammonia per milliliter
		Milligrams
Sugar-beet seed-ball extract (no seeds)	5.79	0.007
Sugar-beet seed-ball extract (50 seeds per dish)	8.23	.254
0.25 percent urea (no seeds)	7.63	.032
0.25 percent urea (50 seeds per dish)	8.61	.443

TABLE 9.—*Analyses of seed-ball extracts and of chemical solutions incubated in glass bottles at 30° C. with a solution prepared from germinating naked sugar-beet seeds*

Source of extract or solution	Incubation period	pH	Nitrogen per milliliter of solution				
			Free NH_3	Combined NH_4^+	Amide N	Residual N	Total N
Sugar-beet variety:	*Days*		*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
U. S. 12-D	0	5.50	0	0.132	0.070	0.542	0.744
	3		.189	.073	.044	.450	.756
	6	7.30	.170	.087	.081	.436	.774
U. S. 12-D (steamed at 20 pounds' pressure for 30 minutes)	2	5.50	.002	.170	.054	.584	.810
	4		.002	.179	.065	.599	.845
	6	5.57	.004	.158	.086	.589	.837
68	0	6.27	0	.119	.171	.355	.645
	3		.301	.023	.053	.269	.646
	6	8.23	.305	.036	.043	.258	.642
Asparagin+1 percent NaCl	0	4.33	0	.019	.317	.378	.714
	3		.324	.318	.010	.074	.726
	6	7.50	.350	.290	.015	.080	.735
Urea+1 percent NaCl	0	5.13	0	.008	.176	.637	.821
	3		.217	.064	.077	.459	.817
	6	8.70	.325	.054	.124	.341	.844

The previous tests indicated the presence of urease, asparaginase, and probably other proteases. The presence of urease in naked sugar-beet seeds was shown by employing the technique described by Annett (*1*). Five grams of naked sugar-beet seeds was ground to a fine

powder and shaken with 50 ml. of distilled water in the presence of toluene for 1½ hours. The mixture was filtered, and 10 ml. of the filtrate was added to 50 ml. of 0.25-percent urea solution. Some of the samples received 0.5 ml. of toluene each; no toluene was added to the others. Five ml. of the mixtures was titrated immediately with 0.035 N hydrochloric acid (HCl) and like amounts at various intervals thereafter over a period of 165 hours. Methyl red was used as the indicator. The results are shown in table 10. It is evident from the data in table 10 that the urease content of sugar-beet seed is very low as compared to that of soybeans, swordbeans, or jackbeans, and that the hydrolysis is decreased by the addition of toluene. The decreased hydrolysis in the presence of toluene may have been due to decreased activity of urease or to slight microbial action in the absence of toluene.

TABLE 10.—*Urease content of naked sugar-beet seeds* [1] *as indicated by the quantities of hydrochloric acid (0.035 N) used in titrations*

Time elapsed from beginning of test (hours)	Hydrochloric acid required with—		
	Urea (toluene check)	Urea+ enzyme	Urea+ enzyme+ toluene
	Milliliters	*Milliliters*	*Milliliters*
0	0	0.10	0.10
5½	0	.42	.40
21	0	1.00	.90
45	0	1.60	1.48
69	0	2.08	1.92
93	0	2.95	2.25
117	0	3.50	2.40
165	0	4.20	2.58
Reaction at 165 hours	*pH* 6.70	*pH* 8.73	*pH* 9.00

[1] 5 ml. of sample titrated with 0.035 N hydrochloric acid; methyl red used as indicator.

pH STUDIES

The ammonia toxicity previously encountered was associated with a rise in pH. Therefore, to evaluate separately the influence of pH and ammonia, further tests were conducted. The changes in the pH of several seed-ball extracts and solutions that were observed during germination tests are shown in table 11. Several attempts were made to stabilize the pH of extracts at low values and the pH of prepared solutions at high values to test the range at which seeds would germinate. Several buffer solutions were tried, none of which successfully stabilized pH during germination. The results of some of the tests are given in table 12. The addition of either citric acid or calcium sulfate to seed-ball extracts retarded the toxic action on germinating seed and permitted greater sprout development. Ultimately, however, the killing of root tips was just as pronounced and the rise in pH nearly as high as when no buffer was used. The effect of germinating seeds on prepared solutions of high initial pH was similar to that already noted on ammonia solutions. The drop in pH of these solutions must have been brought about by the buffering action of organic acids and carbon dioxide liberated during germination, which indicates that the initial stages of germination can proceed

when alkalinity is relatively high. Further tests to determine the influence of alkalinity and of ammonium hydroxide on the germination of seeds and growth of sprouts were made by subjecting the thin cotton substrata on which the seeds were placed to a continuous flow of solutions of definite pH values.

TABLE 11.—*pH values of several sugar-beet seed-ball extracts and of chemical solutions before and after naked sugar-beet seeds had been germinated on them for 7 days*

Source of extract or solution	pH of fresh solution	pH of solutions after seeds were germinated on them	Difference in pH before and after germination
Distilled water	[1] 5.57	6.81	1.24
Sugar-beet variety:			
U. S. 12-A	5.50	7.99	2.49
U. S. 12-B	5.20	7.80	2.60
U. S. 12-C	4.75	7.54	2.79
U. S. 12-D	5.50	8.65	3.15
U. S. 12-D (no seeds)	5.50	[2] 5.79	.29
68	6.27	8.63	2.36
Asparagin and salt	4.33	8.35	4.02
Urea and salt	5.13	8.43	3.30
Urea	7.00	8.55	1.55
$NaNO_3$	5.75	7.22	1.47
$(NH_4)_2SO_4$	5.20	6.48	1.28
0.40 mg. N as NH_3 per ml	10.50	8.40	−2.10
0.20 mg. N as NH_3 per ml	10.20	8.05	−2.15

[1] Average of several measurements during tests; has no significance except to show trend of change during germination tests.
[2] Check to show pH change without germinating seeds.

TABLE 12.—*Influence of buffers on change in pH of extracts and several solutions during germination of naked sugar-beet seeds*

Source of extract or solution	pH of fresh solution	pH of solution after seed had germinated	Germination period	Naked seeds germinated
			Hours	*Percent*
Sugar-beet variety:				
U. S. 12-D. Diluted 1:1 with citric acid solution	4.20	8.78	126	86
U. S. 12-D. Diluted 1:1 with water	5.50	8.83	126	70
U. S. 12-D. Diluted 1:1 with calcium sulfate solution	5.00	8.57	94	82
Sodium hydroxide	8.10	6.70	72	96
	8.80	6.90	72	94
	10.70	7.18	72	96
Sodium carbonate	10.60	8.08	72	86
Calcium carbonate	8.20	6.90	72	94
Disodium phosphate plus sodium carbonate plus tartaric acid	9.25	8.67	88	92
	9.68	9.12	88	88
1-percent disodium phosphate plus sodium hydroxide	8.72	7.75	41	50
	9.76	7.90	41	58
	10.12	8.00	41	46
	10.57	8.20	41	52
	11.12	8.55	41	42
	11.70	9.21	41	8

In a test designed to determine the effect of a maintained pH as obtained by a continuous flow of solution through the substratum, the following method was used. The seeds were placed on the cotton substratum, after which the Petri dishes were covered and sealed with wax, and a flow of about 100 ml. per hour was allowed to drip, through a sealed-in glass tube, on the upper edge of the cotton, then flow through the substratum to the opposite side of the dish, and discharge through a small hole drilled in the bottom of the dish. A small end of the cotton substratum pulled through the hole in the bottom of

the dish served as a wick to remove the excess solution without allowing the entrance of air. The results of these tests (tables 13, 14, and 15) show that seeds will germinate at a fairly high pH on such solutions as monopotassium phosphate (KH_2PO_4) and sodium carbonate (Na_2CO_3), where potassium hydroxide (KOH) was used to raise the pH. Some injury was noticed at the higher pH values on sodium carbonate solutions, but this was slight compared to that produced by ammonia at much lower pH values. The relative effect of the three kinds of solution on germination percentage and the increased effectiveness of maintained concentrations are shown in figure 4.

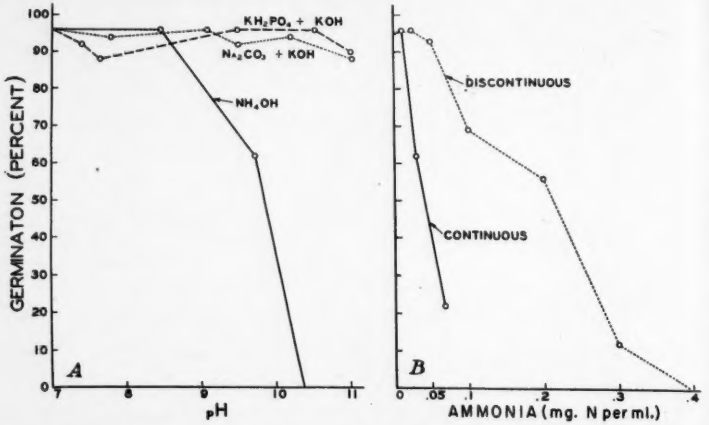

FIGURE 4.—Effect of pH and ammonia concentration (mg. N per ml.) on the germination of naked sugar-beet seeds: *A*, Relative effect of high pH value due to ammonium hydroxide and other bases, in a continuous flow experiment; *B*, relative effect of maintaining the concentration of ammonia by a continuous flow of solution through substratum and of using 9 ml. of solution at beginning and not changing solution.

TABLE 13.—*Influence of a solution of monopotassium phosphate and potassium hydroxide on the germination of cucumber and naked sugar-beet seeds* [1]

Kind of seed	pH of supply solution	20 hours		44 hours	
		Seeds germinated	pH	Seeds germinated	pH
		Percent		*Percent*	
Cucumber seeds	8.30	56	7.37	100	7.18
	9.40	52	7.77	100	6.75
	9.98	41	9.35	92	8.31
	10.68	40	10.52	96	10.12
	11.06	44	10.83	84	11.01
Naked sugar-beet seeds	8.30	54	7.67	88	7.65
	9.40	54	8.18	92	7.40
	9.98	54	9.76	96	9.48
	10.68	51	10.63	96	10.55
	11.06	68	11.03	90	11.01

[1] 1 gm. of KH_2PO_4 per 18 liters of solution; KOH used to raise pH to value noted.

It is also evident from table 15 that the amount of ammonium salt present at a fairly high pH is important. The solution having a pH of 9.31 and containing 0.067 mg. of nitrogen per milliliter showed greater toxicity than the one having a pH of 9.72 but containing only 0.029 mg. of nitrogen per milliliter. The fact that, when the concentration is maintained, a solution containing a very small amount of ammonia may be toxic has apparently not been recognized.

TABLE 14.—*Influence of sodium carbonate solutions at various pH values on the germination of cucumber and naked sugar-beet seeds*

Kind of seed	Na_2CO_3 added to 18 liters of H_2O	pH of supply solution	18 hours		41 hours		68 hours		
			Seeds germinated	pH	Seeds germinated	pH	Seeds germinated	pH	Average length of sprouts
	Grams		*Percent*		*Percent*		*Percent*		*Mm.*
Cucumber seeds	0.3	8.76	84	7.54	92	7.68	96	7.82	17.4
	1.0	9.60	88	8.88	96	9.06	96	9.20	20.4
	2.1	9.96	84	9.35	100	9.58	100	9.57	14.4
	3.0+KOH	10.62	88	9.98	96	10.57	96	10.25	6.5
	3.0+KOH	11.34	88	10.98	100	10.99	100	10.96	5.5
Naked sugar-beet seeds	.3	8.76	66	7.60	94	7.84	94	7.80	6.0
	1.0	9.60	64	8.92	96	9.20	96	9.10	4.6
	2.1	9.96	60	9.44	84	9.48	92	9.52	3.7
	3.0+KOH	10.62	64	10.22	82	10.25	94	10.22	3.0
	3.0+KOH	11.34	52	11.03	68	11.02	88	11.02	2.2

TABLE 15.—*Influence of ammonia solutions at various pH values on the germination of cucumber and naked sugar-beet seeds*

Kind of seed	pH of supply solution	Nitrogen [1]	24 hours		48 hours		72 hours		
			Seeds germinated	pH	Seeds germinated	pH	Seeds germinated	pH	Average length of sprouts
		Milligram	*Percent*		*Percent*		*Percent*		*Millimeters*
Cucumber seeds	8.48	0.014	80	8.05	88	7.94	92	8.07	24.00
	9.31	[2].067	8	9.09	92	9.29	96	9.19	2.44
	9.72	.029	40	9.46	84	9.63	92	9.50	3.48
	10.38	.647	0	10.16	0	10.37	0	10.28	0
Naked sugar-beet seeds	8.48	.014	74	8.27	90	8.38	96	8.18	6.00
	9.31	[2].067	16	9.18	22	9.29	22	9.25	.38
	9.72	.029	28	9.50	52	9.68	62	9.65	1.34
	10.38	.647	0	10.12	0	10.38	0	10.32	0

[1] Measured as milligrams per milliliter of fresh solution.
[2] The pH value of this solution was overstepped; a small amount of phosphoric acid (H_3PO_4) was therefore added and the pH readjusted with NH_4OH.

INFLUENCE OF OSMOTIC PRESSURE AND AMMONIA ON THE GERMINATION OF CERTAIN SEEDS

The toxicity of the water-soluble extract from sugar-beet seed balls is not specific for sugar-beet seed but also affects the germination of several other seeds. Table 16 shows the effect of sugar-beet seed-ball extract on the germination of onion, lettuce, radish, cucumber, cantaloup, tomato, sweet corn, and bean seeds. The germination of some of these seeds was totally inhibited while that of others was merely retarded. There was either an inhibitory or retarding effect on the germination of all kinds of seed on which the extract was tested.

TABLE 16.—*Influence of seed-ball extract of sugar-beet variety 68 on germination of seeds of various plants*

Kind and variety of seed	Treatment	Seeds germinated after—				
		19 hours	45 hours	64 hours	90 hours	138 hours
		Percent	*Percent*	*Percent*	*Percent*	*Percent*
Sugar beet (Variety 68, naked)	Water (check)	56	86	88	88	88
	Extract	0	3	5	5	6
Radish (Scarlet White Tip)	Water (check)	36	60	72	84	90
	Extract	0	2	2	2	2
Lettuce (New York No. 12)	Water (check)	70	84	84	86	88
	Extract	0	0	0	0	0
Cucumber (Klondike White Spine)	Water (check)	66	94	98	98	98
	Extract	0	50	66	[1] 66	66
Onion (Sweet Spanish, or Valencia)	Water (check)	0	58	74	78	82
	Extract	0	0	8	10	10
Tomato (New Stone)	Water (check)	0	60	92	96	96
	Extract	0	0	4	4	4
Cantaloup (Hale Best)	Water (check)	0	58	66	72	76
	Extract	0	0	0	0	0
Sweet corn (Golden Bantam)	Water (check)	0	85	100	100	100
	Extract	0	0	40	75	80
Beans (Red Kidney)	Water (check)	0	5	35	55	70
	Extract	0	0	35	55	65

[1] Tips of radicles barely visible; no subsequent growth.

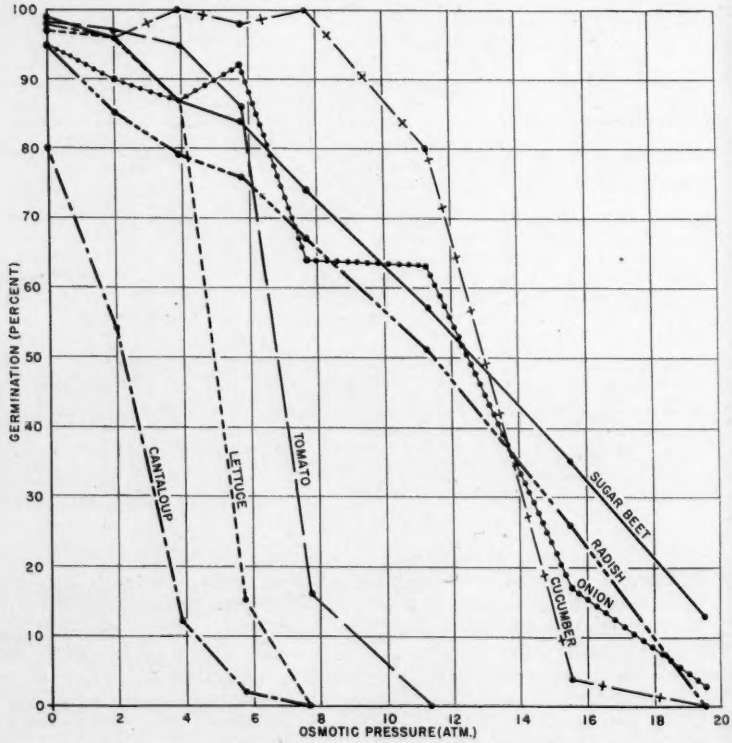

FIGURE 5.—Effect of osmotic pressure on the ultimate germination of several kinds of seeds on a substratum moistened with a sodium chloride solution.

TABLE 17.—*Influence of osmotic pressure on the germination of several kinds of seeds*

[Substratum moistened with water, sucrose, and a sodium chloride solution]

Kind and variety of seed	Solution used	Osmotic pressure	Seeds germinated after—				
			25 hours	49 hours	73 hours	142 hours	257 hours
		Atmospheres	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Radish (Scarlet White Tip)	Water (check)		68	81	88	95	
	Sucrose	2.12	36	57	72	76	
		4.29	25	56	61	67	
		6.12	23	54	62	67	
		8.40	3	47	55	59	
		12.55	1	12	20	26	
		18.53	0	1	2	6	
		23.67	0	0	0	0	
	Sodium chloride	2.00	51	74	83	85	
		3.94	33	56	70	79	
		5.78	31	60	66	76	
		7.73	20	46	59	67	
		11.28	6	17	37	51	
		15.55	0	4	12	26	
		19.54	0	0	0	0	
Lettuce (New York No. 12)	Water (check)		90	94	96	97	
	Sucrose	2.12	71	81	87	92	
		4.29	47	63	72	79	
		6.12	1	16	19	24	
		8.40	0	0	0	0	
	Sodium chloride	2.00	89	94	96	96	
		3.94	55	72	78	87	
		5.78	3	10	11	15	
		7.73	0	0	0	0	
Cucumber (Klondike White Spine)	Water (check)		46	94	96	98	98
	Sucrose	2.12	30	94	96	98	98
		4.29	4	94	100	100	100
		6.12	0	86	98	100	100
		8.40	0	28	70	84	84
		12.55	0	0	0	6	8
		18.53	0	0	0	0	0
	Sodium chloride	2.00	30	96	96	96	96
		3.94	14	90	100	100	100
		5.78	0	92	96	98	98
		7.73	0	66	96	100	100
		11.28	0	0	36	68	80
		15.55	0	0	0	0	4
		19.54	0	0	0	0	0
Onion (Sweet Spanish, or Valencia)	Water (check)		0	15	69	89	95
	Sucrose	2.12	0	1	51	87	92
		4.29	0	2	19	73	88
		6.12	0	0	7	61	78
		8.40	0	0	2	39	72
		12.55	0	0	0	1	16
		18.53	0	0	0	0	2
		23.67	0	0	0	0	0
	Sodium chloride	2.00	0	0	40	80	90
		3.94	0	1	28	77	87
		5.78	0	0	12	83	92
		7.73	0	0	2	39	64
		11.28	0	0	2	19	63
		15.55	0	0	0	1	17
		19.54	0	0	0	0	3
Tomato (New Stone)	Water (check)		0	1	33	94	98
	Sucrose	2.12	0	0	5	78	97
		4.29	0	0	0	19	74
		6.12	0	1	1	1	19
		8.40	0	0	0	0	0
	Sodium chloride	2.00	0	0	0	35	97
		3.94	0	0	0	8	95
		5.78	0	0	0	0	86
		7.73	0	0	0	0	16
		11.28	0	0	0	0	0
Cantaloup (Hale Best)	Water (check)		0	0	16	50	80
	Sucrose	2.12	0	0	2	4	6
		4.29	0	0	0	0	0
	Sodium chloride	2.00	0	0	1	10	54
		3.94	0	0	0	2	12
		5.78	0	0	0	0	2
		7.73	0	0	0	0	0

After it had been demonstrated that there was some differential response of several different kinds of seeds to sugar-beet seed-ball extracts, further tests were conducted to determine the relative tolerance of these seeds to osmotic pressure. The data in table 17 and figure 5 indicate that there was a differential response to osmotic pressure. It is evident that seeds of sugar beet, onion, radish, and cucumber were not so sensitive to osmotic pressure as those of lettuce, tomato, and cantaloup. Furthermore, there was no typical darkening and killing of the sprouts with sucrose and sodium chloride solutions such as had occurred when sugar-beet seed-ball extract was used.

The tolerance of these same seeds to dilute ammonia solutions is shown in table 18. All seeds tested were retarded in germination by concentrations of from 0.2 to 0.4 mg. of nitrogen as ammonia per milliliter. The pH of the substratum was lowered even more than in tests with beet seeds. Further tests showed that the germination of these seeds was completely inhibited when the concentration of free ammonia was maintained, as in the test on cucumber and naked sugar-beet seeds (table 15 and fig. 4).

TABLE 18.—*Toxicity of ammonia as indicated by its power to inhibit the germination of various seeds*

Kind and variety of seed germinated	N as NH_3 per milliliter of fresh solution	pH of fresh solution	pH after germination	Seeds germinated after—		
				48 hours	78 hours	120 hours
	Milligram			*Percent*	*Percent*	*Percent*
Radish (Scarlet White Tip)	[1] None	5.57		60	78	86
	0.1	9.90	4.82	64	88	94
	.2	10.20	4.78	34	58	88
	.3	10.30	4.99	6	50	64
	.4	10.50	5.76	2	38	60
Onion (Sweet Spanish, or Valencia)	[1] None	5.57		58	76	82
	.1	9.90	4.91	24	82	86
	.2	10.20	5.78	24	76	84
	.3	10.30	6.83	4	56	64
	.4	10.50	8.07	2	10	46
Lettuce (New York No. 12)	[1] None	5.57		84	84	86
	.1	9.90	4.81	92	92	92
	.2	10.20	7.50	0	0	28
	.3	10.30	7.73	0	0	0
	.4	10.50	7.83	0	0	0
Tomato (New Stone)	[1] None	5.57		60	94	96
	.1	9.90	5.17	24	78	86
	.2	10.20	5.20	6	86	90
	.3	10.30	6.20	0	66	80
	.4	10.50	6.28	0	22	78
Cantaloup (Hale Best)	[1] None	5.57		58	69	74
	.1	9.90	6.80	0	40	76
	.2	10.20	7.18	0	32	60
	.3	10.30	6.92	0	12	56
	.4	10.50	7.10	0	0	20
Cucumber (Klondike White Spine)	[1] None	5.57	6.10	0	96	100
	.1	9.90	5.95	0	100	100
	.2	10.20	5.72	0	94	98
	.3	10.30	6.65	0	82	96
	.4	10.50	7.31	0	40	98

[1] Water; check solution.

SOME PROPERTIES OF EXTRACTS FROM SEVERAL DRY FRUITS

Studies were conducted to determine whether the physical and chemical properties of water-soluble extracts of other dry fruits were similar to those of sugar-beet seed-ball extracts. Extracts were prepared from the ripened seeds of lettuce, oats, barley, spinach, and celery. Table 19 shows some of the properties of these extracts and

also a comparison of their effect on the germination of both lettuce and naked sugar-beet seeds. It is evident that the extracts from all of the seeds tested had some retarding effect on the germination of both beet and lettuce seeds. With the extracts of barley, oats, spinach, and one of the lots of lettuce seed, this retarding effect on germination was rather small. The extract from celery seeds completely inhibited the germination of lettuce seeds and reduced the germination of naked sugar-beet seeds. The extract from Grand Rapids lettuce seed inhibited the germination of lettuce seeds and reduced the germination of naked sugar-beet seeds more than the sugar-beet seed-ball extract with which it was compared. Extract from Grand Rapids lettuce seeds had a very high total nitrogen content, and a relatively large quantity of ammonia was hydrolyzed from it during germination.

TABLE 19.—*Some properties of water extracts from seeds of lettuce, celery, spinach, oats, barley, and sugar beets as related to toxicity* [1]

Kind of extract	Osmotic pressure	pH of fresh extract	pH of solution after germination of—		Germination of—				Total nitrogen [2]	Free NH_3 [3]
					Naked sugar-beet seeds after—		Lettuce seeds after—			
			Sugar-beet seeds	Lettuce seeds	48 hours	96 hours	48 hours	96 hours		
	Atmospheres				*Percent*	*Percent*	*Percent*	*Percent*	*Milligrams*	*Milligrams*
Water (check)	0.00	5.57	6.81	4.51	90	98	97	99		
Lettuce (New York No. 12)	1.20	5.59	6.27	5.34	88	88	85	88	0.087	0.006
Lettuce (Grand Rapids)		5.69	8.18	8.39	28	44	0	0	1.628	.336
Celery	4.00	5.97	8.37	8.57	60	68	0	0	.261	.012
Spinach	1.45	6.25	8.42	8.52	82	82	90	93	.122	.011
Oats		5.45	7.70	7.65	88	94	51	87	.254	.008
Barley		5.68	7.65	7.90	88	88	80	92	.103	.006
Sugar beet (U. S. 12-D)	4.28	5.68	8.43	7.65	36	50	0	0	.884	.250

[1] Extracts from dry seeds prepared as previously described for sugar-beet seed-ball extracts.
[2] Measured as milligrams per milliliter of fresh solution.
[3] Measured as nitrogen per milliliter of solution after germination.

SOME PROPERTIES OF PRESSED JUICE FROM FLESHY FRUITS OF TOMATOES, CUCUMBERS, AND MELONS

Tests were run to determine the relationship between the properties of dry-seed extracts and the juice from fleshy fruits. Juice was pressed from the ripened fruits of tomatoes, cantaloups, watermelons, and large green cucumbers. The juices were then filtered and sterilized by heating. Several kinds of seeds, whose tolerance to osmotic pressure had previously been determined, were placed on cotton substrata moistened with the pressed juice. Some of the properties of the prepared juices and their effects on the germination of several kinds of seeds are shown in table 20. It is evident that all of the seeds used in the test were retarded in germination, and furthermore that osmotic pressure appeared to be the major factor responsible. This is in contrast to the relative importance of the two major factors responsible for the toxicity of extracts from sugar-beet seed balls and other dry fruits.

Nitrogen analyses were made of fresh juices of fleshy fruits and of the same juices after they had been recovered from substrata on which seeds had been germinated. Similar determinations were made after incubation with an extract prepared from germinating sugar-beet seeds. The average total ammonium nitrogen on five lots of tomato juice immediately after adding 15 ml. of an extract from germinating sugar-beet seeds to 50 ml. of tomato juice was 0.109 mg. per milliliter. After 3 days' incubation this increased to 0.126 mg. and after 6 days' incubation to 0.130 mg. per milliliter. The average pH value on these samples after 6 days' incubation was 4.42. Evidently the buffering capacity (table 20) was sufficiently high to neutralize the free ammonia and render it comparatively ineffective as a toxic agent.

TABLE 20.—*Some properties of pressed juice from the fleshy fruits of tomatoes, cucumbers, and melons, and a comparison of their influence on the germination of seeds with that of salt or sugar solutions of similar osmotic pressure*

Juice or solution used to moisten cotton substratum	Osmotic pressure	Germination of—								pH of fresh juice	Total nitrogen [1]	Sodium hydroxide [2]
		Naked sugar-beet seeds after		Lettuce seeds after		Tomato seeds after		Cucumber seeds after				
		50 hours	72 hours	50 hours	72 hours	50 hours	72 hours	50 hours	72 hours			
	Atmospheres	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*		*Milligrams*	*Milliliters*
Water (check)		90	96	94	99	18	78	94	94			0.1
Tomato juice	8.76	43	54	0	0	0	0	22		4.20	1.048	29.6
Sugar solution [3]	8.40	67	77	0	0	0	0	28	70			
Cucumber juice	5.77	72	80	0	16	2	2	91	92	5.77	.377	4.4
Salt solution [3]	5.78	69	78	10	11	0	0	92	96			
Cantaloup juice	15.33	16	32	0	0	1	1	0	0	6.01	1.112	7.0
Salt solution [3]	15.55	21	34	0	0	0	0	0	0			
Watermelon juice	12.73	28	32	0	0	0	0	0	0	5.41	.481	3.7
Sugar solution [3]	12.55	40	62	0	0	0	0	0	0			

[1] Measured as milligrams per milliliter of fresh solution.
[2] Milliliters of 0.035 normal NaOH required to raise 10 milliliters of pressed juice to pH 9; indicates relative buffering capacity of the pressed juice.
[3] Data taken from earlier experiment on the effect of osmotic pressure; therefore direct comparison is not possible, but general trend is very obvious.

DISCUSSION

In the rather extensive investigations that numerous workers have made on the factors affecting the germination of seeds in fleshy fruits, the only factor that has received common recognition is osmotic pressure. At various times such indefinite terms as unnamed "specific inhibitors" and "metabolic byproducts of germination" have been postulated as sole or contributory factors adversely affecting germination.

In the present paper the effects of osmotic pressure and other factors affecting seed germination in the presence of water extracts have been evaluated. Although osmotic pressure may be the major factor causing inhibition of germination by fruit juices, evidence is here presented that, in the case of water extracts from the seed envelopes of dry fruits, free ammonia released from nitrogenous compounds in the course of seed germination is a more significant factor.

A summary of the relative importance of the principal factors on which the toxicity of sugar-beet seed-ball extracts was found to be dependent is graphically presented in figure 6. The data, which were taken from table 5, show that free ammonia, pH value, and toxicity are correlated in all cases except one, that of the U. S. 12–C lot grown on extremely saline soil. The very high osmotic pressure in this case was probably a more important factor than the free ammonia. This case also indicates the magnitude of difference in osmotic pressure necessary to depress germination. There was an extreme difference

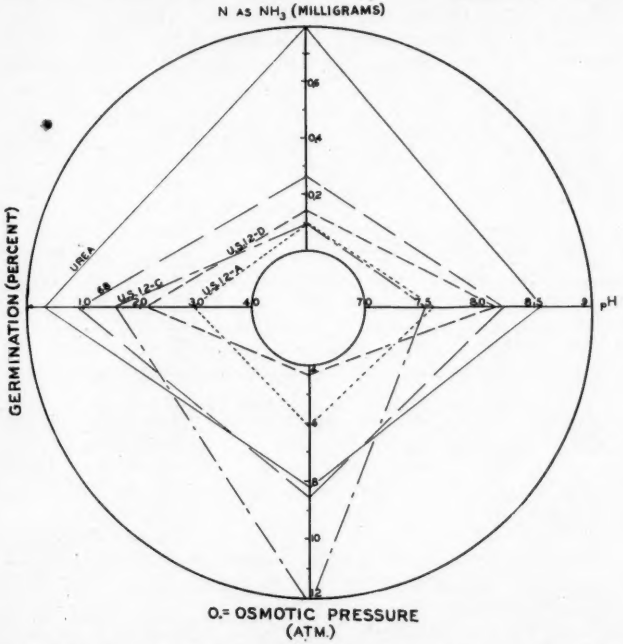

FIGURE 6.—Relation of free ammonia, hydrogen-ion concentration, and osmotic pressure to the toxicity of sugar-beet seed-ball extracts and a solution of urea. Lines drawn from the points on the axes serve to aid interpretation of correlation between factors. The circles indicate boundaries of the figure.

between the osmotic pressure of seed from U. S. 12–D and that of seed from U. S. 12–C. The lack of direct relationship between toxicity and osmotic pressure is indicated by the crossing of the lines in the diagram between the percentage germination and osmotic pressure axes.

Sufficient ammonia was released by germinating seeds from water extracts of sugar-beet seed balls and certain other dry fruits to account adequately for the toxic effects that have been observed when seeds are germinated in the presence of these extracts. The ammonia was hydrolyzed from the water-soluble nitrogen compounds of seed extracts by both germinating seeds and solutions prepared from germinating seeds. The ammonia released not only neutralized organic

acids produced during germination but also caused a sharp rise in the pH of the substratum. In the case of sugar-beet and certain lettuce-seed extracts, 0.3 to 0.4 mg. of nitrogen as ammonia per milliliter of solution was released. Synthetic solutions containing a similar amount of ammonia were shown to be toxic to germinating seeds. Even smaller amounts of ammonia were toxic when the ammonia solution was allowed to flow continuously through the substratum so that the concentration was not reduced by neutralization.

The toxicity of seed extracts and synthetic organic nitrogen solutions was governed not only by the amount of hydrolyzable nitrogen present but also by the rapidity of hydrolysis. Thus asparagin, which was more slowly hydrolyzed than urea, was less toxic than urea during the early stages of germination. Since the hydrolytic agent was present in the immediate vicinity of the seed, the concentration of ammonia was greatest near the seed and the concentration increased as germination progressed. The greater tolerance of radish and cucumber seeds to ammonia solutions was shown to be due more to the production of organic acids than to an actual tolerance to larger amounts of free ammonia. That free ammonia was the toxic agent rather than ammonium salts was shown by the relatively slight inhibition produced by fairly high concentrations of ammonium sulfate. A solution of ammonium sulfate containing 3.5 mg. of nitrogen per milliliter reduced germination less than did a solution of ammonium hydroxide containing 0.3 mg. of nitrogen per milliliter. Still lower concentrations of ammonium hydroxide were effective when the concentration was maintained by a continuous change of solution (fig. 4, *B*).

The present studies showed that ammonia was toxic not only to germinating sugar-beet seeds but also to seedlings and plants that were 2 months old. This is in line with the conclusion of Willis and Rankin (*26*, *27*), who reported that ammonia released from concentrated organic nitrogenous fertilizer applied to the soil was toxic to cotton seedlings. The conclusion of Willis and Rankin was challenged by Tiedjens and Robbins (*24*), who claimed that plants grew as well when ammonium hydroxide was used as the sole source of nitrogen as when some other form such as ammonium sulfate or calcium nitrate was used. However, the pH of the nutrient solutions of Tiedjens and Robbins was evidently very unstable. Starting with an initial pH as high as 9.0 in a continuous-flow experiment, the highest pH value recorded on the solution after passing through the sand culture was 3.8. It is evident that very little free ammonia was maintained in contact with the plants.

Fife and Frampton (*5*) reported that when sugar-beet plants were subjected to high concentrations of carbon dioxide there was a distinct rise in the pH of the juice extracted from the leaf blades owing to the liberation of ammonia from amides and other soluble nitrogenous compounds. They further demonstrated that the amount of ammonia released was dependent on the original nitrogen content of the leaves.

The toxicity of seed extracts varied between species and varieties and between different seed lots of the same variety. This variation was influenced by several factors such as the amount of nitrogen present in the extracts, the rapidity with which it could be hydrolyzed to ammonia, and the buffering capacity of the seed extract. This

latter factor was found to be of special importance in the case of fleshy-fruit juices and will be discussed more fully later.

Among the factors that influence nitrogen content of seed are nitrogen nutrition and genetic constitution. Pucher, Curtis, and Vickery (*17*) have shown that betanin, the red pigment of the beet, contains approximately 6 percent of nitrogen. Keller (*8*) has shown that color differences in beets are due to differences in genetic constitution, although environmental factors may also play a part in determining how the genetic factors are expressed. One of the very apparent characteristics of sugar-beet variety 68 was its comparatively high red pigmentation of seed pericarp, which may be the source of some of the nitrogenous compounds that are water soluble and from which ammonia is hydrolyzed during seed germination.

The relation of color to nitrogen content may be more important in the case of lettuce varieties. Of the varieties studied, seed of the Grand Rapids variety was conspicuous by its black color and high nitrogen content. Data reported in this paper indicate that at least twice as much free ammonia was released from the water extract of lettuce seed of the Grand Rapids variety as was necessary greatly to retard or inhibit the germination of lettuce seed in Petri dishes. Knowledge of the fact that relatively large amounts of free ammonia are released from some lettuce-seed extracts offers an explanation for the phenomena reported by Shuck (*20*, *21*). Shuck found that when lots of seed of some varieties of lettuce are successively germinated on the same substratum by products of germination accumulate which have an inhibitory action on germination. He stated that when these substances have accumulated to a point at which no more seeds will germinate the substratum can then be washed and seeds will again germinate normally. It is evident that during the germination of successive lots of seed on a moist substratum soluble nitrogen compounds would accumulate so that eventually there would be a condition similar to one where the cotton was moistened with seed extract.

Thompson (*22*) reported that some varieties of lettuce are hard to germinate but that others are germinated readily. He stated that seed of the Grand Rapids variety is generally hard to germinate and gave data to show that this difficulty could be largely overcome by both soaking and drying the seed at low temperature. It is evident that the removal of water-soluble nitrogen during the soaking process would minimize any damage that otherwise might result from the release of ammonia by the germinating seed. Thompson also presented data to show that certain conditions of light and temperature following the soaking or washing treatment further increased germination. The effect of light and temperature may be entirely different from the effect of washing and may have no connection with prevention of damage from free ammonia. Later, Thompson and Kosar (*23*) reported several sulfur compounds such as thiourea, thiosemicarbazide, etc., as stimulating the germination of dormant lettuce seed. The comparable compounds containing oxygen in place of sulfur or hexavalent sulfur such as sulfate were either ineffective or toxic to germination. The nature of the chemicals that stimulated germination, all of which contained divalent sulfur, suggests the possibility that they may have influenced the amount of free ammonia present by either

retarding enzymatic hydrolysis or by chemical combination. Tests conducted by the authors have shown that sugar-beet seed balls previously soaked in a solution of thiourea germinate more uniformly and show less injury after long periods of cold storage, such as are used in vernalization tests, than with any other treatment thus far tried. In tests with sugar-beet seed-ball extracts, where the principal inhibiting factors are known to be hydrolyzed free ammonia and osmotic pressure, the addition of thiourea, although it increased the osmotic pressure, reduced the toxic effect, as shown by increased germination percentage and improved appearance of the sprouts of the test seeds.

Much of the inhibitory effect of tomato, cucumber, and melon juice on the germination of sugar-beet, tomato, lettuce, and cucumber seed can be accounted for by the osmotic pressure of the juice. Lavialle (*10*) and Litvinov (*12*) stated that the essential cause of the inhibitory action of all fruit juices is osmotic pressure. When seeds were germinated on a substratum moistened with tomato juice, the low initial pH of the juice and its high buffering capacity prevented the release of free ammonia. Some nitrogenous compounds in the tomato juice were hydrolyzed during germination tests, but the high acidity of the tomato juice held the ammonia in a combined form and prevented it from being a major factor in germination inhibition. Litvinov stated that the active acidity of the tomato juice was not responsible for any inhibiting action, but that on the contrary an increase in inhibition occurred when the pH of the juice was raised to 6.5. It is easy to see that a rise in the initial pH of the tomato juice might make the release of ammonia a more important factor. In fact, tests conducted by the authors showed that tomato juice increased in toxicity as the pH was raised and that sugar-beet seed-ball extracts decreased in toxicity when they were buffered at a low pH.

Ammonium hydroxide solutions seemed to have some effect on germination other than that accounted for by a rise in pH, even though the rise in pH of the substratum on which seed had germinated corresponded rather closely with the extent of injury. It was shown that ammonium sulfate at a low pH was not very toxic, and also that seeds were able to initiate germination and subsequently neutralize sodium hydroxide or sodium carbonate solutions having an initial pH between 11 and 12. By using a continuous flow of fresh solution through the substratum on which seeds were placed and protecting the solutions from any neutralizing influences of gases such as carbon dioxide, it was possible to determine the effect of a maintained pH. The data showed that ammonium hydroxide at pH 9.3 to 9.7 was more toxic to germination and growth than either potassium phosphate or sodium carbonate at a pH of more than 11. Ammonia solutions at a pH slightly above 10 completely inhibited germination. The data also showed that at pH values above 9 the amount of ammonium ion available for release as free ammonia may also be a critical factor in the germination of seeds.

SUMMARY

Studies are reported relating to the properties of dry- and fleshy-fruit extracts that are responsible for toxic and inhibitory effects on germinating seed.

In the case of sugar-beet seed-ball extracts, the toxic effect on germinating seed was shown to be largely due to the toxic action of ammonia, which was released from the nitrogenous compounds of the extracts by enzymatic hydrolysis.

The presence of from 0.3 to 0.4 mg. of free ammonia per milliliter of solution was toxic to germinating seeds, young seedlings, and 2-month-old sugar-beet plants.

Sufficient ammonia was released not only from sugar-beet seed-ball extracts but also from certain other dry-fruit extracts and solutions of urea and asparagin to account for their toxic effect on germinating seeds.

Although the ammonia released from sugar-beet seed balls and some lettuce-seed extracts was sufficient to cause a rise of 4 units in the pH of the solutions, the increase in pH did not completely explain the toxic action of the released ammonia. This was shown by the fact that ammonium hydroxide solutions at pH 9 to 9.5 were more toxic to germinating seeds than either monopotassium phosphate or sodium carbonate solution at pH 11 to 11.5.

The tolerance of various seeds to ammonia solutions and osmotic pressure is reported. There was a marked differential tolerance of the seeds tested to osmotic pressure, but all seeds were sensitive to ammonia solutions containing 0.1 mg. of nitrogen per milliliter when the ammonia concentration was maintained.

In contrast to the major role played by ammonia in the toxic action of dry-fruit extracts, osmotic pressure seemed largely to account for the inhibition to germination of seeds in contact with juices from fleshy fruits. This difference is explained on the basis of the high acid-buffering capacity of fruit juices, which prevented the accumulation of ammonia in the free state. Combined ammonia in the form of salts was shown to be comparatively nontoxic.

The removal of water-soluble nitrogen fractions from the pericarpal tissue affords an explanation of the beneficial effects of washing or soaking some seeds prior to germination tests.

LITERATURE CITED

(1) Annett, Harold Edward.
1914. the urease content of certain indian seeds. Biochem. Jour. 8: [449]–452.

(2) Axentjev, B. N.
1927. ueber die wirkung von samenauszügen auf das keimen von samen. Russ. Bot. Obshch. Zhur. (Soc. Bot. de Russie, Jour.) 12 (3): 291–306. [In Russian, German summary, pp. 305–306.]

(3) Borriss, H.
1936. über das wesen der keimungsfordernden wirkung der erde. Deut. Bot. Gesell. Ber. 54: 472–486. illus.

(4) Borthwick, H. A., and Robbins, W. W.
1928. lettuce seed and its germination. Hilgardia 3: [275]–304, illus.

(5) Fife, J. M., and Frampton, V. L.
1935. the effect of carbon dioxide upon the ph and certain nitrogen fractions of the sugar-beet plant. Jour. Biol. Chem. 109: 643–655, illus.

(6) Fukaki, Sadayoshi.
1930. über die frage der beeinflussung des eigenen fruchtsaftes auf die samenkeimung. Kyushu Imp. Univ., Dept. Agr. Bul. Sci. (Jour. Agr. Sci.) [Jap. title: Nogei Zasshi] 4: 119–133. [In Japanese. German summary, pp. 132–133.]

(7) HENDRICKS, R. H., THOMAS, M. D., STOUT, MYRON, AND TOLMAN, BION.
—— ABSORPTION AND CONDUCTIMETRIC DETERMINATION OF AMMONIA IN BORIC ACID SOLUTION AND ITS APPLICATION TO NITROGEN DISTRIBUTION STUDIES. Indus. and Engin. Chem., Analyt. ed. [In press.]
(8) KELLER, WESLEY.
1936. INHERITANCE OF SOME MAJOR COLOR TYPES IN BEETS. Jour. Agr. Res. 52: 27–38.
(9) KÖCKEMANN, ALFONS.
1934. ÜBER EINE KEIMUNGSHEMMENDE SUBSTANZ IN FLEISCHIGEN FRÜCHTEN. Deut. Bot. Gesell. Ber. 52: 523–526.
(10) LAVIALLE, PIERRE.
1929. OBSERVATIONS ET CONSIDÉRATIONS SUR LA GERMINATION ACCIDENTELLE DES GRAINES AU SEIN DES FRUITS CHARNUS. Soc. Bot. de France Bul. 76 (3/4): 276–279.
(11) LEHMANN, WERNER.
1937. ZUR KEIMUNGSPHYSIOLOGIE DES BUCHWEIZENS. Landw. Jahrb. 84: 771–778, illus.
(12) LITVINOV, L. S.
1938. ON THE CAUSES OF THE INHIBITING EFFECT OF TOMATO SAP ON THE GERMINATION OF TOMATO SEEDS. Perm. Biol. Nauch. Issled. Inst. (Inst. des Rech. Biol. de Perm.) Bul. 11 (5–6): 163–171. [In Russian. English summary, p. 171.]
(13) MOSHEOV, GERSHON.
1938. THE INFLUENCE OF THE WATER EXTRACT OF WHEAT SEEDS UPON THEIR GERMINATION AND GROWTH. (Note) Palestine Jour. Bot., Jerusalem, Ser. 1 (1): 86–92.
(14) OPPENHEIMER, HEINZ.
1923. KEIMUNGSHEMMENDE SUBSTANZEN IN DER FRUCHT VON SOLANUM LYCOPERSICUM UND IN ANDEREN PFLANZEN. Akad. der Wiss. Wien, Math.-Nat. Kl. Sitzber. Abt. 1, 131: [59]–65.
(15) ———
1923. DAS UNTERBLEIBEN DER KEIMUNG IN DEN BEHÄLTERN DER MUTTERPFLANZE. Akad. der Wiss. Wien, Math.-Nat. Kl. Sitzber. Abt. 1, 131: [279]–312, illus.
(16) ORCUTT, F. S., AND WILSON, P. W.
1936. BIOCHEMICAL METHODS FOR THE STUDY OF NITROGEN METABOLISM IN PLANTS. Plant Physiol. 11: 713–729, illus.
(17) PUCHER, GEORGE W., CURTIS, LAWRENCE C., AND VICKERY, HUBERT BRADFORD.
1938. THE RED PIGMENT OF THE ROOT OF THE BEET (BETA VULGARIS). I. THE PREPARATION OF BETANIN. II. A METHOD TO DETERMINE BETANIN. Jour. Biol. Chem. 123: 61–75.
(18) REINHARD, A. W.
1933. ZUR FRAGE DER SAMENKEIMUNG BEI SOLANUM LYCOPERSICUM. Planta 20: [792]–794.
(19) SESSIONS, A. C., AND SHIVE, J. W.
1928. A METHOD FOR THE DETERMINATION OF INORGANIC NITROGEN IN PLANT EXTRACTS. Plant Physiol. 3: 499–511, illus.
(20) SHUCK, A. L.
1934. SOME FACTORS INFLUENCING THE GERMINATION OF LETTUCE SEED IN SEED LABORATORY PRACTICE. N. Y. Agr. Expt. Sta. Tech. Bul. 222, 21 pp., illus.
(21) ———
1935. A GROWTH-INHIBITING SUBSTANCE IN LETTUCE SEEDS. Science (n. s.) 81: 236.
(22) THOMPSON, ROSS C.
1938. DORMANCY IN LETTUCE SEED AND SOME FACTORS INFLUENCING ITS GERMINATION. U. S. Dept. Agr. Tech. Bul. 655, 20 pp.
(23) ——— AND KOSAR, WILLIAM F.
1939. STIMULATION OF GERMINATION OF DORMANT LETTUCE SEED BY SULPHUR COMPOUNDS. Plant Physiol. 14: 567–573, illus.
(24) TIEDJENS, V. A., AND ROBBINS, W. R.
1931. THE USE OF AMMONIA AND NITRATE NITROGEN BY CERTAIN CROP PLANTS. N. J. Agr. Expt. Sta. Bul. 526, 46 pp., illus.

(25) TOLMAN, BION, AND STOUT, MYRON.
1940. TOXIC EFFECT ON GERMINATING SUGAR-BEET SEED OF WATER-SOLUBLE SUBSTANCES IN THE SEED BALL. Jour. Agr. Res. 61: 817–830, illus.
(26) WILLIS, L. G., AND RANKIN, W. H.
1930. FREE-AMMONIA INJURY WITH CONCENTRATED FERTILIZERS. Indus. and Engin. Chem. 22: 1405–1407, illus.
(27) ——— AND RANKIN, W. H.
1932. IS AMMONIUM HYDROXIDE TOXIC TO COTTON PLANTS? Science (n. s.) 76: 214–215.

EFFECT OF CERTAIN ENVIRONMENTAL CONDITIONS ON THE PREVALENCE OF OPHIOBOLUS GRAMINIS IN THE SOIL[1]

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INTRODUCTION

Investigations of fungi and bacteria in soil have shown that the type and number are affected greatly by environment. The effect of environmental factors on the prevalence of soil-borne parasites has received but little attention, in contrast to the extensive studies of the environmental factors affecting the behavior of parasitic organisms in pure culture or in tests of pathogenicity. In the case of soil-borne parasites, information on the behavior in infested soils under various conditions is more important than that on the causal organisms in pure culture.

The experiments reported here were made to determine whether certain environmental conditions, particularly temperature, moisture, and compactness of the soil, favored the retention of *Ophiobolus graminis* Sacc., the parasite that causes take-all of wheat, in naturally infested uncropped soils. For comparison, a few experiments on the tolerance of this organism to extreme temperatures were conducted in pure culture.

EXPERIMENTAL METHODS

In the studies of *Ophiobolus graminis* in pure culture, low temperatures were obtained by both outdoor exposure and artificial refrigeration. Cultures of *O. graminis* were grown on potato-dextrose agar in test tubes. Certain of these cultures were kept in an ordinary thermograph shelter, and others were buried at progressive depths in the soil, where they were exposed to decreasing extremes of temperature. The temperatures were measured with maximum and minimum thermometers and a thermograph. At various intervals during the winter transfers were made to find out whether the fungus was viable.

The thermal death point of *Ophiobolus graminis* growing on potato-dextrose agar was determined by the standard method employed by bacteriologists, with modifications for fungi.

In the tests of infested soils the samples were exposed to various temperatures by outdoor exposure in flats, in the greenhouse in flats and metal pans, and in a freezing chamber in flats; or they were stored in a deep cave in cans. Soil samples described as "moist" were maintained at approximately 60 percent of the maximum water-

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holding capacity, while the dry samples were completely air-dried. Looseness of the soil was maintained by occasional stirring, and compactness by thorough tamping while in a moist condition.

The severity of take-all lesions on Kanred wheat grown in the greenhouse was used as an indication of the presence and extent of infestation in a given sample of soil. The method has been described previously.[2]

EXPERIMENTAL RESULTS

EFFECT OF EXTREME TEMPERATURES ON OPHIOBOLUS GRAMINIS IN PURE CULTURE

Davis [3] showed that *Ophiobolus graminis* grows best at about 23° to 24° C. At 4° growth was exceedingly slight, while at 36° it was checked entirely. The fact that the fungus grows only slightly at 4° C. (39.2° F.) suggested the experiments to determine whether the winter cold in Kansas might not only check its growth but possibly kill it. These experiments were conducted during the winter of 1925–26 and 1926–27.

All the cultures were viable at the end of both winters. The weekly maximum and minimum temperatures in the instrument house, given in table 1, show that the organisms survived temperatures as low as −2° F. Cultures of the fungus also were subjected to repeated alternations of temperature ranging from 70° to as low as −20°. None of the exposures, shown in table 2, affected the viability of the fungus.

Determinations of the thermal death point of *Ophiobolus graminis* showed that both the microhyphae and the macrohyphae were killed when exposed for 10 minutes at 122° F. (50° C.). The fungus remained viable after exposure for 10, 20, and 30 minutes to a temperature of 113° F. (45° C.). Cultures exposed 30 minutes recovered more slowly than those exposed for shorter periods.

TABLE 1.—*Weekly minimum and maximum outdoor temperatures during winter months of 1926 and 1927 when cultures of Ophiobolus graminis were stored outdoors at Manhattan, Kans.*

Year	Month	Experiment No.	Temperature							
			First week		Second week		Third week		Fourth week	
			Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
			° F.	° F.	° F.	° F.	° F.	° F.	° F.	° F.
1926	January	1					54	3	52	0
1926	February	1	56	24	63	20	59	1	68	28
1926	March	1	58	14	70	14	78	20		
1926	December	2			55	−1	48	12	57	16
1927	January	2	64	16	48	−2	36	9	64	14
1927	February	2	62	30	52	5	72	7	70	23
1927	March	2	66	9	68	25	77	23	66	25
1927	April	2	77	30						

[2] FELLOWS, HURLEY, and FICKE, C. H. SOIL INFESTATION BY OPHIOBOLUS GRAMINIS AND ITS SPREAD. Jour. Agr. Res. 58: 505–519, illus. 1939.

[3] DAVIS, RAY J. STUDIES ON OPHIOBOLUS GRAMINIS SACC. AND THE TAKE-ALL DISEASE OF WHEAT. Jour. Agr. Res. 31: 801–825, illus. 1925.

TABLE 2.—*Time and temperature of exposure in a freezing chamber of pure cultures of Ophiobolus graminis*

Period of freezing		Temperature			Period of freezing		Temperature		
Date	Duration	Minimum	Maximum	Mean	Date	Duration	Minimum	Maximum	Mean
1926	*Hours*	° *F.*	° *F.*	° *F.*	*1927*	*Hours*	° *F.*	° *F.*	° *F.*
Dec. 3–4	5.0	[1] −13	10.4	−8.7	Jan. 6–7	16.5	[1] −13.0	30.0	9.5
Dec. 7–8	29.0	[1] −20.2	15.8	−1.1	Jan. 8–15	81.0	[2] −11.2	30.2	9.0
Dec. 9–10	20.5	[1] −16.6	21.2	−1.3	Jan. 18–22	38.0	[1] −7.6	30.2	7.8
Dec. 11–12	2.0	[1] −16.6	−4.0	−11.3	Jan. 31	9.0	[1] −5.8	22.1	4.1
Dec. 13–14	5.5	[1] −13.9	1.4	−5.9	Feb. 10–11	16.5	[1] −5.8	26.6	−.4
Dec. 16–17	25.0	[1] −12.1	21.2	−6.1	Feb. 14–15	11.5	[1] −7.6	−.4	−2.9
Dec. 20–21	9.5	[1] −9.4	10.4	−1.8	Feb. 17	8.5	[1] −2.2	23.0	6.3
					Feb. 19	7.0	[1] −.4	24.8	6.3

[1] Cultures were held at a temperature of approximately 70° F. during the intervals between freezing.
[2] Cultures kept below freezing while the freezing chamber was not operating.

EFFECT OF EXTREME TEMPERATURES AND DROUGHT ON OPHIOBOLUS GRAMINIS IN SOIL

Numerous experiments were conducted in several years to determine whether summer heat and drought would reduce the quantity of *Ophiobolus graminis* in infested soil in the field. These experiments were made by comparing the relative abundance of the fungus in the upper soil layers with that in the deeper layers, which had been subjected to less heat and drought.

The severity tests with Kanred wheat showed that the fungus was reduced somewhat during the summer in the upper 2-inch stratum, but the difference in the abundance of the organism between this stratum and lower depths down to 10 inches was too small to be of practical importance. All the soil strata studied retained sufficient infestation to produce severely diseased wheat plants.

Infested soils were exposed to more severe conditions in the greenhouse. In 1929, 1931, and 1932, soil from infested spots in the field was taken to the greenhouse in the spring, spread in 1-inch layers in shallow pans, and exposed unshaded and without watering until the latter part of October. In 1932 the tests were duplicated with soil samples placed in an elevated position outdoors, where they were protected from the rain but exposed to the sun during the summer. Control samples were stored in a cool cave. The soils were tested for infestation during the winter following the exposure. The results are given in table 3, and temperatures of the soil samples exposed outdoors in 1932 are shown in figure 1. The temperatures of the soil samples stored in the greenhouse are not given, but they were much lower than those of the samples exposed outdoors.

All the soil samples exposed to heat and drought retained enough infestation to produce severely diseased plants, but in three of the four tests the infestation was reduced to some extent. The experiment performed in 1931 showed that soil kept in the greenhouse had more infestation than the control. However, in this experiment the severity rating was high, and when plants are very severely diseased it is difficult to make fine distinctions. Consequently, the apparent difference may not be very significant

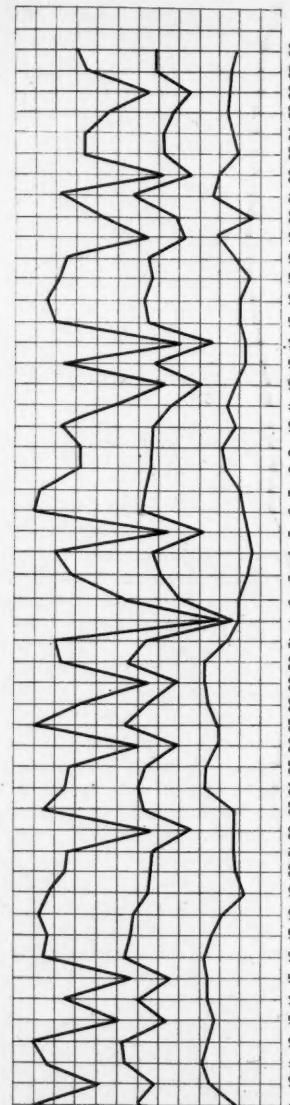

FIGURE 1.—Maximum, minimum, and average daily temperatures of a layer of infested soil 1 inch thick exposed outdoors during August and September 1932.

TABLE 3.—*Severity of take-all on wheat plants grown in the greenhouse in infested soil that had been dried in the greenhouse or outdoors, Manhattan, Kans., 1929-32*

Soil treatment and year	Tests	Plants	Average height of plants	Plants tillering	Culms		Plants dead	Plants having diseased—			Roots retained	Average severity of lesions on—			Severity rating
					Number	Heading		Crowns	Culms	Roots		Crowns	Culms	Roots	
Dried in greenhouse:	*No.*	*No.*	*In.*	*Pct.*		*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	
1929	6	105	25.5	48.2	195	9.5	36.1	45.4	25.9	42.1	78.0	67.5	60.0	66.7	20.8
1931	8	70	17.5	27.1	92	8.6	40.0	100.0	96.1	99.7	20.1	90.7	76.8	92.1	148.5
1932	8	59	23.8	13.8	65	35.6	14.8	100.0	32.1	99.6	54.0	69.8	52.4	67.0	48.8
Average[1] and total	22	234	22.2	29.7	352	17.5	30.3	81.8	51.3	80.4	50.7	76.6	63.0	75.5	72.7
Dried outdoors:															
1932	6	55	22.8	50.9	101	44.5	34.5	64.5	48.8	64.1	57.4	77.8	61.8	85.3	38.3
Controls:[2]															
Infested soil:															
1929	6	111	20.2	7.2	124	23.3	28.8	84.9	55.5	79.6	48.3	87.0	74.4	81.4	66.6
1931	9	70	16.2	31.4	118	16.1	44.2	100.0	82.4	98.9	23.5	90.0	67.1	83.2	115.1
1932	4	33	19.5	21.2	40	12.5	6.0	109.0	52.4	99.6	45.0	84.0	36.5	78.0	63.2
Average[1] and total	19	214	18.5	19.9	282	17.5	26.5	94.9	63.4	92.7	38.9	87.0	59.3	80.8	80.4
Sterilized soil:															
1929	6	90	28.4	30.0	140	59.2	0	0	0	0	100.0	0	0	0	0
1931	10	86	30.6	82.5	249	53.8	0	0	0	0	100.0	0	0	0	0
1932	6	54	30.8	98.1	218	72.0	0	0	0	0	100.0	0	0	0	0
Average[1] and total	22	230	29.9	70.2	607	61.6	0	0	0	0	100.0	0	0	0	0

[1] Simple averages.
[2] Soil collected from the same spot but not exposed to heat and drought

Infested soil exposed alternately to growing and freezing temperatures under conditions identical with those of the organism in pure culture showed no reduction of the disease on wheat plants subsequently grown in the soil. Thus, alternate freezing and thawing did not injure the organism in either soil or pure culture. The temperature records are shown in table 2.

In an experiment in which *Ophiobolus graminis* was grown in pure culture it had a thermal death point of 122° F. (50° C.) when exposed for 10 minutes. In another experiment in soil it was not killed at temperatures that ranged from 140° to 160° F. for more than an hour on each of several days. The fact that the macrohyphae on infected plants are much coarser and their walls thicker than in pure culture may explain this difference.

EFFECT OF TEMPERATURE, MOISTURE CONTENT, AND COMPACTNESS OF SOIL ON VIABILITY OF OPHIOBOLUS GRAMINIS

The effect of exposure conditions on *Ophiobolus graminis* either in the absence or in the presence of the host should be reflected later on wheat plants grown in the soil.

The three environmental factors most likely to affect soil flora in the field are moisture, temperature, and compactness or tilth. Accordingly soil from infested spots in the field was exposed for various lengths of time to eight combinations of these three factors. These

combinations were: (1) moist, cool, compact; (2) dry, cool, compact; (3) moist, cool, loose; (4) dry, cool, loose; (5) moist, warm, compact; (6) dry, warm, compact; (7) moist, warm, loose; and (8) dry, warm, loose.

The two temperature conditions were obtained by storing one set of soil samples in a deep cave and the other in the greenhouse. The temperature in the cave averaged 37.5° F. and was always above freezing during subzero weather, and in the summer it averaged 71.5°, never going above 72°. The greenhouse temperature was fairly uniform during the winter, with an average of 70.2°, and was variable during the summer but averaged 83.6°. The average maximum daily temperature in June was 97.1°, in July 110.7°, and in August 102.5°. The dry compact samples were so thoroughly puddled and hard at the end of the storage period that a hammer was required to pulverize

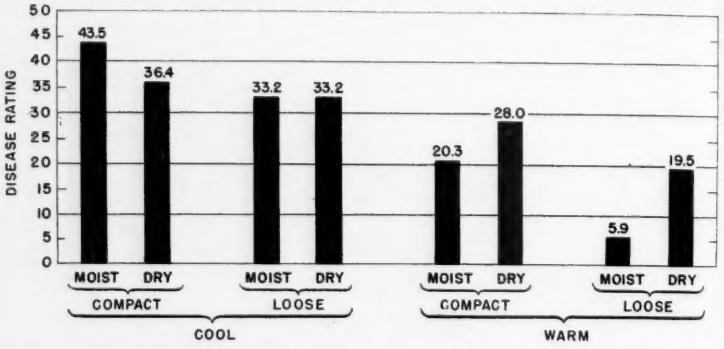

FIGURE 2.—Average severity ratings of take-all on wheat plants grown in the greenhouse in infested soil stored previously under different conditions of moisture, temperature, and compactness, at Manhattan, Kans., 1930–36. (Data in table 4.)

them. The dry samples were air-dried. Water was applied to the moist samples from time to time. At the end of the storage period all of the soil samples were brought to favorable tilth and moisture, and wheat was grown in them in the greenhouse.

This experiment was conducted four times during the period from 1930 to 1936. For a given experiment all the soil was taken from the same infested spot in the field and was well mixed. Tests for infestation in the first experiment were on plants grown in soil stored for 230 days; in the second experiment, 338 days; in the third, 777 days; and in the fourth, 608 days.

The data on the severity of take-all on the wheat plants grown in the greenhouse in the various soil samples are given in table 4, and the average severity ratings are shown graphically in figure 2. The data in table 4 are summarized in table 5 to show the effects of the environmental factors considered separately and in combinations of two. The relative importance of the factors is indicated by averages and totals from the combinations into which they entered. The severity ratings shown in table 5 are presented graphically in figure 3.

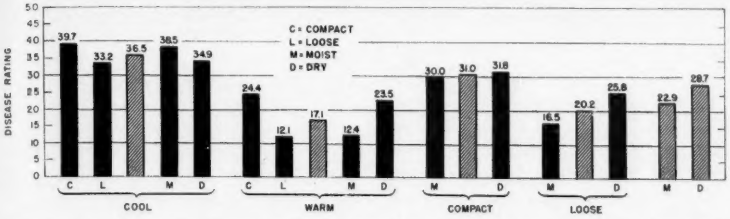

FIGURE 3.—Disease-severity ratings showing the relative effect of single and double environmental factors on the retention of *Ophiobolus graminis* in stored infested soil. The hatched columns represent weighted averages for each single factor. (Data in table 5.)

Under the conditions of the experiments the storage temperature had more effect on the viability of *Ophiobolus graminis* in infested soil than had any other factor. Cool temperature favored the fungus regardless of the other factors. Warm soil combined with other factors in any manner retained less infestation than any similar combination with a cool soil.

TABLE 4.—*Severity of take-all on wheat plants grown in the greenhouse in infested soil previously stored under different conditions of moisture, temperature, and compactness, Manhattan, Kans., 1930–36*

[The length of storage in the various experiments was as follows: No. 1, 230 days; No. 2, 338 days; No. 3, 777 days; and No. 4, 608 days]

Condition of soil in storage	Experiment No.	Tests	Plants	Average height of plants	Plants tillering	Culms			Plants dead	Plants having diseased—			Roots retained	Average severity of lesions on—			Severity rating
						Number	Heading	Average number per tillering plant		Crowns	Culms	Roots		Crowns	Culms	Roots	
Cool, compact:		*Number*	*Number*	*Inches*	*Percent*		*Percent*		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Moist	1	4	36	37.2	68.9	99	43.4	.....	0	25.5	15.8	25.4	90.7	58.3	11.6	43.5	10.9
	2	5	46	11.4	43.4	69	0	2.15	36.9	100.0	87.1	99.7	15.2	80.9	44.2	98.3	143.8
	3	5	58	24.0	29.3	81	27.1	2.35	0	100.0	24.5	89.0	49.0	60.4	9.6	70.0	37.9
	4	6	47	16.3	29.7	69	5.7	2.56	12.7	100.0	77.3	98.8	50.8	79.8	45.0	75.0	58.8
Average and total	.....	20	187	22.2	42.8	318	19.0	.....	12.4	81.3	51.1	78.2	51.4	69.8	27.6	71.7	[1] 43.5
Dry	1	3	27	34.3	44.4	59	16.9	.....	14.8	100.0	88.8	95.3	35.3	79.6	67.3	84.2	71.2
	2	6	58	17.0	58.6	108	5.0	2.47	37.9	100.0	98.1	99.9	19.7	92.3	70.0	95.3	109.4
	3	5	61	24.4	32.7	88	31.8	2.35	1.6	58.5	4.4	52.4	86.0	50.1	68.3	31.2	15.0
	4	6	52	27.3	21.1	68	66.1	2.45	3.8	16.6	7.4	15.8	91.6	47.7	43.7	75.0	8.1
Average and total	.....	20	198	25.7	39.2	323	29.9	.....	14.5	68.7	49.6	65.8	58.1	67.4	62.3	71.4	[1] 36.4
Cool, loose:																	
Moist	1	3	28	35.5	50.0	52	17.3	.....	0	16.6	0	6.1	99.1	16.5	0	37.7	4.4
	2	5	56	16.4	28.5	78	2.5	2.37	19.6	100.0	76.0	97.5	18.5	78.5	53.6	89.7	136.5
	3	4	40	23.0	47.5	62	30.6	2.15	0	100.0	0	84.7	74.0	60.1	0	56.7	21.8
	4	6	51	14.3	19.6	61	0	2.00	15.6	100 0	58.8	98.1	62.5	75.4	39.8	58.3	52.1
Average and total	.....	18	175	22.3	36.4	253	12.6	.....	8.8	79.1	33.7	72.1	63.5	57.6	23.3	60.6	[1] 33.2
Dry	1	2	21	39.0	66.6	67	26.8	.....	0	20.5	0	2.0	100.0	9.0	0	32.3	3.3
	2	5	46	20.6	52.1	84	9.5	2.58	10.2	100.0	87.1	99.4	41.2	74.3	41.2	78.8	61.7
	3	4	46	19.2	17.3	55	21.0	2.12	2.1	100.0	16.3	94.5	52.9	76.1	11.4	60.1	42.3
	4	6	50	17.1	8.0	54	25.9	2.00	18.0	93.9	65.3	90.3	56.6	72.0	49.5	75.0	51.8
Average and total	.....	17	163	23.9	36.0	260	20.8	.....	7.5	78.6	42.1	71.5	62.6	57.8	25.5	61.5	[1] 33.2

Warm, compact:																		
Moist	1	4	37	40.0	72.9	159	26.4	------	0	0	0	.2	100.0	0	0	35.0	1.1
	2	5	52	10.4	34.6	76	0	2.22	38.4	100.0	85.9	99.9	25.9	83.6	62.8	89.4	124.0
	3	5	52	37.2	44.2	88	64.7	2.60	0	25.0	7.5	20.1	90.1	40.0	18.3	56.1	8.6
	4	6	55	21.0	34.5	82	26.8	2.42	1.8	50.0	16.6	49.1	76.6	75.0	23.8	68.7	18.9
Average and total	------	20	196	27.1	46.5	405	29.8	------	10.0	43.7	27.5	42.3	73.1	49.6	26.2	62.3	[1] 20.3
Dry	1	2	15	27.5	60.0	31	22.5	------	20.0	81.2	50.0	52.2	53.8	62.0	86.4	71.5	37.0
	2	6	60	15.1	65.0	136	13.2	2.94	33.3	100.0	91.8	100.0	21.3	74.5	64.8	93.3	85.6
	3	5	60	29.0	36.6	91	57.1	2.40	0	42.0	0	16.1	94.6	36.3	0	30.3	7.8
	4	6	42	26.1	42.8	64	28.1	2.22	0	57.7	26.4	39.6	84.4	34.0	42.5	59.2	13.8
Average and total	------	19	177	24.4	51.1	322	30.2	------	13.3	70.2	42.0	51.9	63.5	51.7	48.4	63.5	[1] 28.0
Warm, loose:																		
Moist	1	3	33	39.3	60.6	90	52.2	------	0	0	0	0	100.0	0	0	0	0
	2	5	39	18.0	100.0	182	24.7	4.66	7.6	74.2	42.5	73.0	50.9	66.2	57.6	62.9	27.5
	3	3	33	33.0	15.1	39	87.1	2.20	0	5.5	0	.6	100.0	5.0	0	2.0	−.8
	4	6	50	23.8	30.0	78	26.8	2.84	0	0	0	Trace	100.0	0	0	0	−25.6
Average and total	------	17	155	28.5	51.4	389	47.7	------	1.9	19.9	10.6	18.4	87.7	17.8	14.4	16.2	[1] 5.9
Dry	1	3	29	41.6	79.3	104	31.5	------	0	30.0	3.3	22.1	90.6	7.7	75.0	59.6	8.7
	2	5	48	16.8	56.2	95	17.8	2.74	35.4	100.0	83.3	99.8	27.3	93.6	75.8	89.3	83.2
	3	3	32	32.0	34.3	45	66.6	2.18	0	18.1	0	2.0	100.0	2.0	0	2.0	1.5
	4	5	41	22.2	26.8	59	30.5	2.63	0	54.5	32.4	44.8	80.0	61.4	30.0	59.7	19.1
Average and total	------	16	150	28.1	49.1	303	36.6	------	8.8	50.6	29.7	42.1	74.4	48.6	45.2	52.6	[1] 19.5

[1] Calculated from averages, not an average of severity ratings. For method see footnote 2, p. 724.

TABLE 5.—*Summary of data from table 4 to show the weighted average influence of single environmental factors and of two environmental factors on the prevalence of Ophiobolus graminis in infested soil*

Condition of soil in storage	Tests	Plants	Average height of plants	Plants tillering	Culms		Plants dead	Plants having diseased—			Roots retained	Average severity of lesions on—			Final severity rating
					Number	Heading		Crowns	Culms	Roots		Crowns	Culms	Roots	
Single environmental factor: [1]	*Number*	*Number*	*Inches*	*Percent*		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
Cool	75	723	23.5	38.2	1,154	20.5	10.8	76.9	44.1	71.9	58.9	63.1	34.6	66.3	36.5
Warm	72	678	27.0	49.5	1,419	36.0	8.5	40.1	27.4	38.6	74.6	42.0	33.5	48.6	17.1
Compact	79	758	24.8	44.5	1,368	27.3	12.5	65.9	42.5	59.5	61.5	59.7	41.1	67.2	31.0
Loose	68	643	25.7	43.2	1,205	29.4	6.7	57.0	29.0	51.0	72.0	45.4	27.1	47.7	20.2
Moist	75	713	25.0	43.9	1,305	27.2	8.2	56.0	30.7	52.7	68.9	48.7	22.8	52.7	22.9
Dry	72	688	25.5	43.8	1,208	29.3	11.0	67.0	40.8	57.8	64.6	56.5	45.3	62.2	28.7
The two environmental factors: [2]															
Cool:															
Compact	40	385	23.9	40.3	641	24.0	13.4	75.0	50.3	72.0	54.7	68.6	44.9	71.5	39.7
Loose	35	338	23.1	36.2	513	16.7	8.1	78.8	37.9	71.8	63.0	57.7	24.4	61.0	33.2
Moist	38	362	22.2	28.9	571	15.3	10.8	80.2	42.4	75.1	57.4	63.7	25.4	66.1	38.5
Dry	37	361	24.8	37.6	585	25.3	11.0	73.6	45.8	68.6	60.3	62.6	43.9	66.4	34.9
Warm:															
Compact	39	373	25.7	43.8	727	29.8	11.6	56.9	34.7	47.1	68.3	50.6	37.3	67.9	24.4
Loose	33	305	28.3	50.2	692	42.1	5.3	35.2	20.1	30.2	81.0	33.2	29.8	34.4	12.1
Moist	37	351	27.8	48.9	794	38.5	5.9	31.8	19.0	30.3	80.4	33.7	20.3	39.2	12.4
Dry	35	327	26.2	50.1	625	33.4	11.0	60.4	35.8	47.0	68.9	50.1	46.8	58.0	23.5
Compact:															
Moist	40	383	24.6	44.0	723	24.2	11.2	62.5	39.3	60.2	62.2	59.7	26.9	67.0	30.0
Dry	39	375	25.0	45.1	645	30.0	13.9	69.4	45.8	58.8	60.8	59.0	55.3	67.4	31.8
Loose:															
Moist	35	330	25.4	43.9	642	30.1	5.3	49.5	22.1	45.2	75.6	37.7	18.8	38.4	16.5
Dry	33	313	26.0	42.5	563	28.7	8.1	69.6	35.4	56.8	68.5	53.2	35.3	57.0	25.8

[1] Averages and totals derived from all the combinations into which the following single factor entered.
[2] Averages and totals derived from all the combinations into which the following combinations of two factors entered.

Both moisture and compactness modified the effect of temperature on the prevalence of *Ophiobolus graminis* in infested soil. When the infested soil was cool and loose, moisture appeared to be of little importance, because the plants grown in the cool loose soil that had been stored in either a dry or a moist condition had equal disease ratings. A compact warm soil retained more of the fungus in a viable state than a loose one. The addition of moisture to warm soil, either loose or compact, tended to rid the soil of infestation. However, the effect of moisture was greater when the soil was loose.

The individual experiments are all in agreement in indicating that temperature is the most important factor influencing the retention of the parasite in the soil. Some of the results of experiment 1, however, are at variance with those of other experiments with regard to moisture and compactness. This is due perhaps to the fact that the soil in experiment 1 was stored for a much shorter period than in the other experiments.

Infestation did not increase under any condition of storage. In experiments 2, 3, and 4 additional soil was taken to the greenhouse at the time the soil was collected, and wheat was sown at once in order to compare the disease produced initially with that after the soils had been stored. Since the highest retention of infestation was shown to have occurred in the soils stored in a cool condition, this group is used for comparison with the initial infestation in table 6. It is evident that even cool uncropped soil lost some infestation, as might be expected, since Sewell and Melchers [4] have shown that rotation of crops is an aid in controlling the take-all disease.

TABLE 6.—*Comparison of the presence of Ophiobolus graminis in portions of the same infested soil sample, one portion fresh and the other stored*

Experiment No.	Severity rating					
	Stored soil					Fresh soil
	Cool, compact		Cool, loose		Average	
	Moist	Dry	Moist	Dry		
2	143.8	109.4	136.5	61.7	112.8	115.1
3	37.9	15.0	21.8	42.3	29.2	63.2
4	58.8	8.1	52.1	51.8	42.7	93.6
Average	80.1	44.1	70.1	51.9	61.5	90.6

DISCUSSION

The soil is a common medium for many micro-organisms. The interactions of these organisms may be mutually deleterious, helpful, or neutral. Consequently the results obtained in studies of the effect of soil environment on a single organism are difficult to interpret because of the likelihood of indirect effects of other organisms. Thus the results with *Ophiobolus graminis* just described might have been different if the infested soil had contained a somewhat different microflora.

[4] SEWELL, M. C., and MELCHERS, L. E. THE EFFECT OF ROTATION AND TILLAGE ON FOOT-ROT OF WHEAT IN KANSAS, 1920–1924. Amer. Soc. Agron. Jour. 16: 768–771, illus. 1924.

Aside from the effect of a varying microflora, the effect on the parasite of fluctuations of temperature and moisture within a single year or season should not be great when the infested soil is cropped to a susceptible plant, because an abundance of the host during the growing season provides an opportunity for growth of the fungus to offset the effects of adverse weather conditions. However, there might be a gradual reduction of the organism during a cycle of dry, hot years, or an accelerated accumulation during a cool, moist cycle.

It would seem that adverse weather conditions should have the maximum effect on the fungus in infested soil planted to a nonsusceptible crop. Under these conditions hot weather should reduce infestation, the fungus might not be rehabilitated even though cool weather occurred later, and infestation would diminish from year to year.

SUMMARY

Ophiobolus graminis Sacc., the parasite causing take-all of wheat, when in pure culture was not killed by the winter temperatures occurring in Kansas, nor was it affected by repeated abrupt alternations of growing and subfreezing temperatures. The thermal death point of both microhyphae and macrohyphae was about 122° F. (50° C.).

In naturally infested soil the fungus survived temperatures as high as 160° F. (71° C.). High temperatures and drought in the summer reduced the infestation in infested soils only slightly. The abundance of the fungus in infested soils was not reduced by repeated alternations from growing to subfreezing temperatures.

The abundance of the parasite in infested soil was reduced in different degrees by different combinations of moisture, temperature, and compactness of the soil during storage. However, the fungus remained viable in either moist or dry infested soil stored in a warm greenhouse as long as 777 days. In general, cool soil tended to retain the fungus longer than warm soil. The fungus was reduced most in a warm, loose, moist soil, and least in a cool, compact, moist soil.

CHEMICAL COMPOSITION OF SYMPHORICARPOS ROTUNDIFOLIUS AS INFLUENCED BY SOIL, SITE, AND DATE OF COLLECTION[1]

By L. A. STODDART[2]

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INTRODUCTION

For a number of years the Utah Agricultural Experiment Station conducted an experiment on the feeding of mineral supplement to range cattle on mountain summer pastures which involved the collection of many of the more important range plant species at regular intervals to determine their chemical composition and thus permit the calculation of the mineral content of the animal diet. Collections of plant material were made at regular calendar intervals, since it was necessary to correlate plant composition with animal reaction as measured at regular time intervals by weight and blood composition. The importance of stage of maturity as a factor in chemical composition was recognized, but, in such heterogeneous range, developmental variations between species and within species made it difficult even to define the growth stage existent at the time of plant selection.

Sampling of vegetation was accomplished by means of a single collection on each collection date. The samples were composited and thus were representative of many areas within the 400-acre pasture. These areas were not regular stations but merely places where the collector happened upon the particular species being collected from the heterogeneous plant cover. Data obtained after several years of such sampling were so variable as to cause some question as to their accuracy. Because of this concern, a sampling experiment was devised to study variability in chemical composition attributable to the following factors: (1) Date of collection, (2) soil type, (3) site or habitat, (4) field sampling technique, and (5) laboratory analysis technique.

LITERATURE REVIEW

Studies correlating natural variation in chemical composition with habitat factors are rare, but there are many papers dealing with seasonal trends and with response to fertilizers.

Of the papers dealing with seasonal trend, a recent report on California foothill range plants is typical. Gordon and Sampson (*6*)[3] showed for herbaceous plants as the season progressed an orderly decline in crude protein, silica-free ash, calcium, phosphorous, and potassium. Crude fiber increased regularly. Deciduous shrubs as the season progressed showed a decline in protein, potassium, and phos-

[1] Received for publication December 15, 1940. Contribution of the Range Management Department, Utah Agricultural Experiment Station; report on Project 162.

[2] Acknowledgment is hereby made of the indispensable assistance given by Dr. A. E. Brandt of the Soil Conservation Service, U. S. Department of Agriculture, in the design and analysis of this experiment, and of the services of the Department of Bacteriology and Biochemistry of the Utah Agricultural Experiment Station in the chemical analyses.

[3] Italic numbers in parentheses refer to Literature Cited, p. 738.

phorus but an increase in silica-free ash and calcium, and little change in crude fiber.

Stanley and Hodgson (*13*) found regular declines in crude protein and phosphorus as the season progressed and increases in nitrogen-free extracts of Arizona grasses.

Studies in Oklahoma (*4*) showed a close relationship between precipitation and the calcium-phosphorus balance in *Andropogon scoparius*. As precipitation increased calcium content decreased and phosphorus content increased, and as precipitation decreased calcium increased and phosphorus decreased.

Hopper and Nesbitt (*9*) found a quadratic relationship between the April, July, and October composition of grass hay; the July yield being higher in protein and nitrogen-free extract, and lower in fiber. A similar relationship for crude fiber was found in Arizona grasses (*13*).

Orr (*11*), in his classic work on plant minerals, reports positive correlation between mineral content, especially phosphorus, and soil moisture, which, of course, varies with the season. Season influences minerals in that they are highest during periods of maximum leafiness and activity of protoplasm.

Fagan and Milton (*5*) decided that the composition of grasses varied with season largely because of a different leaf-to-stem ratio. McCreary (*10*) suggested that late season increases in total ash and lime might be attributed to dust accumulations.

The response of plant composition to soil was investigated by Truninger and Grünigen (*14*), who studied the relation between soil reaction and phosphorus, potassium, and calcium but found no correlation.

Holtz (*8*) found in oats and certain clovers a relationship between phosphorus and calcium content and the soil upon which they were grown. Peculiarly, the phosphorus content of oats followed the total phosphorus content of the soil, while that of red clover followed more closely the available phosphorus content of the soil. In some soils, calcium content of oats followed the calcium content of the soil but in others no relationship was found.

Orr (*11*) concluded that mineral composition within a species is determined primarily by the soil as shown by the response to fertilizers.

Watkins (*15*) found that *Bromus inermis* when grown in the sun was high in percentage of carbohydrates and low in percentage of nitrogen as compared to plants grown in the shade. Long-day plants as compared to short-day plants were high in percentage of carbohydrates and low in percentage of nitrogen. At bloom stage nitrogen-fertilized plants as compared to check plants were high in percentage of carbohydrate and low in percentage of nitrogen.

Greaves (*7*) found a highly significant positive correlation between total ash and calcium, and total ash and magnesium; between calcium and magnesium; and between phosphorus and crude protein, and phosphorus and crude fat. He found a highly significant negative correlation between total ash and phosphorus, and total ash and calcium and crude fiber and crude fat; between calcium and phosphorus and nitrogen-free extract; and between crude fiber and phosphorus, and crude protein. Similar results have been found in other studies (*3*).

Daniel (*3*), in studies of certain native and cultivated grasses and legumes in Oklahoma, showed positive genetic relationships in the

chemical composition of the plants. Thus, the content of plants that are normally low in calcium and phosphorus remained so when the plants were grown on soils rich in these minerals and, conversely, plants normally high in calcium and phosphorus remained so when grown on soil poor in these minerals.

AREA OF STUDY

The investigations described herein were made on high mountain pastures east of Logan, Utah, a region lying within the aspen-Douglas fir zone. The topography is very steep and irregular, the soils highly variable, and the vegetation correspondingly complex. The pastures were not grazed during the season of study.

SOILS

Although no official soil survey has been made of this range-pasture area, the soils were mapped by standard soil survey methods for the purpose of this experiment. Eighteen distinct types were delimited, differing in texture, depth, color, and other profile characters. These types have not been officially described nor given series and type names. Geologically this area is composed of residual sandstones and limestones of the Wasatch conglomerate formation. In many areas the formation has been washed very thin and a substratum of dolomite of the adjacent Nounan formation is very close to the surface.

Three of the more important soil types were selected for use in this study. The basis for selection was the quantity of lime present in the soil as determined by the hydrochloric acid test. Although this was the basis for the selection, numerous other differences were apparent some of which may have been as important as the lime content. It should likewise be noted that total lime content may not be an index to available calcium. Brief descriptions of the three soils, designated A, B, and C, follow.

Soil A.—This type has a surface layer of limefree black soil high in gravel and 3 inches in depth. This layer is underlain to a depth of 72 inches by a red clay high in calcium carbonate. The soil was differentiated from all others by the presence of large quantities of lime in the upper layers, and though occasionally the upper portions of the clay are free from calcium, this is not usual. The subsoils are free from gravel.

Soil B.—This type has a surface layer of black silty clay loam containing some gravel and extending to a depth of 12 to 18 inches. A red clay layer is found under the black surface. Gravel and stones are abundant in local areas but are not common, and abundant calcium is found only in the deep layers beginning at 42- to 54-inch depths.

Soil C.—This soil type is a brownish-black, gravelly, silty clay loam to depths of 12 to 18 inches below which there is a layer of red to yellowish-brown gravelly clay. No calcium carbonate is present, though in local areas limestone was found.

VEGETATION

The vegetation of the pasture belongs chiefly to the sagebrush type or to the aspen type, and both types are of complex composition. In order to eliminate the variable factor of plant-cover type, all sampling

was done in the sagebrush type. This type (fig. 1) was analyzed in detail by sample plots and found to have a density (foliage cover) of 20.7 percent. Grasses formed 7.0 percent of the foliage cover; browse plants, 71.1 percent; and forbs, 21.9 percent. A detailed analysis of the composition of the foliage cover follows.

Grasses:	Percent	Forbs:	Percent
Agropyron pauciflorum	2.0	*Achillea lanulosa*	2.2
Bromus tectorum	2.5	*Agastache urticifolia*	1.3
Stipa columbiana	.5	*Aster* spp	.8
Others	2.0	*Balsamorhiza sagittata*	.5
Browse plants:		*Cogswellia macrocarpa*	.7
Amelanchier alnifolia	3.7	*Collinsia tenella*	1.2
Artemisia tridentata	24.4	*Crepis acuminata*	1.3
Chrysothamnus viscidiflorus	13.9	*Eriogonum heracleoides*	3.4
Prunus melanocarpa	6.7	*Geranium fremontii*	1.2
Purshia tridentata	7.7	*Lupinus caudatus*	2.5
Symphoricarpos rotundifolius	14.5	*Senecio serra*	.5
Others	.2	*Stellaria jamesiana*	1.4
		Vicia americana	.5
		Helianthella uniflora	1.7
		Wyethia amplexicaulis	1.5
		Others	1.2

The grazing season on this area as prescribed by the Forest Service of the United States Department of Agriculture is from about July 1 to October 15.

CLIMATIC CONDITIONS

The climatic conditions of the area studied are typical of those in most of the western mountains. Heavy snows remain until about May 1 and the summers are comparatively dry but cool, resulting in good growing conditions throughout a normal summer. The annual precipitation, though not measured, was estimated at 20 inches.

The year during which these studies were made was abnormally dry, adjacent stations showing annual precipitation to be only one-half to two-thirds of normal. There was very little rain during the growing season. Rainfall in May and June was normal, but in July it was only about one-half of normal and all occurred after the samples were collected. Precipitation during August was negligible. September, however, was a near-normal month, with heavy rains about 1 week before the collections were made.

METHODS

Because of the complexity of the vegetation, one species, the round-leaf snowberry (*Symphoricarpos rotundifolius*), a palatable and abundant shrub, was selected for study. This plant was rather uniformly distributed over all soil types and, hence, lent itself well to the study.

It is doubtful whether the reactions of this species would be typical of all other species or, indeed would remain constant for this species from year to year. It was felt, however, that data from this species could be used as an index to the importance that should be accorded a particular factor in collecting plants for analysis.

Collections were made on 3 days in the summer of 1939, July 15, August 15, and September 15. Since normal fruiting did not take place (presumably because of a late frost) it was impossible to describe the stage of maturity except to state that the first collection was

considered about full-leaf growth and the last date marked the beginning of leaf dropping.

In addition, from each soil type samples were taken from two sites or habitats, called good and poor. The good sites were simply areas of vigorous or above-average growth, often north exposures or areas where moisture conditions were better. Poor sites were often dry southern exposures and growth was less vigorous. Topography also influenced the difference in growth through concentration of moisture. The difference between the two was not sharp, but, in the field, it was obvious. Variation between these sites could be attributed in part

FIGURE 1.—Sagebrush type upon which collections were made. The aspen type is visible in the background.

to a difference in developmental stage and, in this respect, might be analogous to seasonal differences within a site.

The method of collecting material for analysis was designed particularly to eliminate personal error by the following procedure. Transects were considered to be 100 paces along predecided lines. On each soil and site at each date numbers were drawn to determine at which paces along the transect plant material would be collected. Duplicate collections were made within each 100-pace transect. At two points thus selected, and these were marked "a" and "b" in the order of collection. All "a" collections and all "b" collections from 10 such transects were composited, each constituting exactly one-tenth of the total sample. In collecting, the closest plant to the selected point was harvested, all current growth being included.

Thus at each of three collection dates (early, medium, late) duplicate samples were collected from 3 soil types (A, B, and C), and from each soil type on 2 sites (good, poor), giving a total of 12 separate samples at each date or 36 for the season. Each of these samples was, of course,

a composite of collections from 10 subareas. Chemical analyses were made in duplicate without regard to their checking, and each analysis was recorded separately to make possible the calculation of an analysis error. Standard analyses (*1*) were made for total ash, calcium, magnesium, phosphorus, crude protein, crude fat, crude fiber, and nitrogen-free extract. On the whole the experiment was set up on the basis of a factorial design. Although there were only 2 direct replications, there was considerable hidden replication, and as a result each conclusion is founded upon at least 12 replications.

Data were studied by the analysis of variance. Individual sums of squares were computed for each degree of freedom (*12, p. 336*). This method of computing is somewhat empirical and involves selected comparisons and, hence, cannot be expressed by a standard equation. In result, it does not differ from the common methods of determining variance except that it permits a subdivision of the variance according to source.

As an example, let us suppose X_1, X_2, and X_3 to be the three observed variables and it is desired to compare the first with the second and the first two with the third. The two comparisons would be represented thus:

$$\frac{(X_1-X_2)^2}{1^2+1^2} \tag{1}$$

$$\frac{(X_1+X_2-2X_3)^2}{1^2+1^2+2^2} \tag{2}$$

The resultant sums of squares are divided by the degrees of freedom to obtain mean squares for each comparison (see table 4).

Through the use of this method it was possible to measure tendencies toward either linear or quadratic relationships between composition and date of collection and between composition and soils. Since but three points were determined for each curve, the data merely measure general trends. For general tests of significance the F table of Snedecor (*12*) was used, and unless otherwise specified, interactions were grouped to form the error term.

ANALYTICAL DATA

PROTEIN

A summary of the results of the protein analyses is presented in table 1. Protein showed a highly significant [4] decline as the season progressed, the variation with time being strongly linear.[5] The decline was from an average of 11.24 percent on July 15 to 9.19 on August 15 and to 6.58 percent on September 15. Date of collection was far more important than any other factor in its influence upon protein. Site generally had a significant, though slight, effect upon protein, the good sites yielding an average of 9.31 percent and the poor an average of 8.69 percent. This relationship ceased to exist in late season, however, for there was no significant difference between the two sites in September.

[4] An effect or difference is said to be significant if it is of such magnitude that the probability of its chance occurrence in undifferentiated material is equal to or less than 0.05. If the probability is equal to or less than 0.01 the effect is highly significant.

[5] Mean square values are shown in table 4.

TABLE 1.—*Chemical composition (air-dry-weight basis) of snowberry plants collected on three different dates, as affected by soil type and site; data based on an average of four analyses*

PROTEIN CONTENT

Collection date	Composition of plants grown on—						Average
	A soil		B soil		C soil		
	Good site	Poor site	Good site	Poor site	Good site	Poor site	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
July 15	11.99	10.67	12.41	10.83	11.11	10.45	11.24
Aug. 15	9.75	8.47	9.78	9.55	9.00	8.57	9.19
Sept. 15	6.71	6.49	6.45	6.47	6.63	6.77	6.58
Average	9.48	8.54	9.55	8.95	8.91	8.60	
Average for both sites	9.01		9.25		8.75		9.00

NITROGEN-FREE-EXTRACT CONTENT

July 15	47.13	48.56	48.87	50.04	46.89	49.47	48.49
Aug. 15	47.88	48.89	45.84	47.77	47.88	47.45	47.62
Sept. 15	43.40	43.83	42.02	43.81	44.17	44.13	43.56
Average	46.14	47.09	45.58	47.21	46.31	47.02	
Average for both sites	46.61		46.39		46.66		46.56

CRUDE-FIBER CONTENT

July 15	16.20	15.37	13.85	13.83	16.56	14.09	14.98
Aug. 15	16.43	18.29	18.44	17.02	17.80	16.38	17.39
Sept. 15	25.13	26.14	27.36	25.76	25.34	22.77	25.42
Average	19.25	19.93	19.88	18.87	19.90	17.75	
Average for both sites	19.59		19.37		18.82		19.26

CRUDE-FAT CONTENT

July 15	11.16	11.80	11.18	12.13	11.21	12.07	11.58
Aug. 15	11.96	11.20	12.21	12.27	11.70	12.64	12.00
Sept. 15	11.76	11.14	12.32	11.56	11.34	13.44	11.93
Average	11.63	11.38	11.90	11.99	11.42	12.72	
Average for both sites	11.50		11.95		12.07		11.84

TOTAL ASH CONTENT

July 15	6.65	6.59	6.79	6.48	7.41	7.06	6.83
Aug. 15	6.85	6.42	6.65	6.26	6.75	7.61	6.76
Sept. 15	5.41	5.15	5.10	5.18	5.83	5.27	5.32
Average	6.30	6.05	6.18	5.97	6.66	6.65	
Average for both sites	6.18		6.07		6.66		6.30

PHOSPHORUS CONTENT

July 15	0.234	0.289	0.301	0.266	0.372	0.252	0.286
Aug. 15	.150	.201	.208	.178	.297	.429	.244
Sept. 15	.147	.163	.156	.151	.295	.181	.182
Average	.177	.218	.222	.198	.321	.287	
Average for both sites	0.197		0.210		0.304		.237

TABLE 1.—*Chemical composition (air-dry-weight basis) of snowberry plants collected on three different dates, as affected by soil type and site; data based on an average of four analyses*—Continued

CALCIUM CONTENT

Collection date	Composition of plants grown on—						Average
	A soil		B soil		C soil		
	Good site	Poor site	Good site	Poor site	Good site	Poor site	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
July 15	1.43	1.52	1.36	1.37	1.31	1.48	1.41
Aug. 15	1.51	1.72	1.61	1.52	1.62	1.63	1.60
Sept. 15	1.39	1.51	1.36	1.32	1.32	1.59	1.41
Average	1.44	1.58	1.44	1.40	1.42	1.57	
Average for both sites	1.51		1.42		1.49		1.47

MAGNESIUM CONTENT

July 15	0.319	0.424	0.331	0.390	0.318	0.368	0.358
Aug. 15	.262	.302	.303	.309	.288	.293	.293
Sept. 15	.295	.328	.250	.286	.245	.316	.287
Average	.292	.351	.295	.328	.284	.326	
Average for both sites	0.321		0.311		0.305		.313

There was an insignificant quadratic relationship between soil and protein, the B soil being more favorable for protein than either the A or the C soil. This relationship was especially obvious in the August analysis but entirely absent in the September analysis. However, the interaction was not significant.

NITROGEN-FREE EXTRACT

Date of collection was far more important than any other factor in its influence upon nitrogen-free extracts. The pronounced decline as season progressed was significantly greater during the last month than during the first month (table 1); in fact, in the A soil there was a slight increase between July and August.

Nitrogen-free extract bore no significant relation either linear or quadratic to soil type; the soil types responded differently at different dates, the B soil appearing to have a greater seasonal response than either the A or the C soil. Site had a significant, though not large, influence, the average percent being 46.01 on good sites and 47.10 on poor sites.

CRUDE FIBER

A very highly significant increase was found in crude fiber as the season progressed and, though the relationship appears to be linear, there was a greater increase during the last month than during the first (table 1).

An insignificant positive relationship existed between lime content of soil and crude fiber yield from plants collected on poor sites, the relationship being essentially linear.

Analysis brings to light an interesting interaction between date of collection and soil in their influence upon crude fiber. The B soil

was much more reactive to date than either the A or the C soil. Plants from the B soil contained less fiber at the first analysis and more at the last than those from either of the other soils. A slightly greater fiber content was found in plants from good sites (19.68 percent) than in plants from poor sites (18.56 percent).

CRUDE FAT

Crude fat did not differ significantly with changes in soil, date, or site. However, the data indicated a close relationship between fat content of forage and soil type (table 1). This was especially evident on poor sites.

A small but not quite significant relationship existed between site and fat content of the forage. Poor sites averaged 12.03 percent fat and good sites 11.65 percent.

TOTAL ASH

The total ash content was found to decrease as the season progressed, the decrease being significantly greater in the last month than in the first (table 1).

There was likewise a highly significant relation between soil and total ash content, the C soil averaging more than either the A or the B soil.

Site was insignificantly related to ash content, the poor site averaging 6.22 percent and the good site 6.38 percent.

PHOSPHORUS

Phosphorus showed a highly significant relation to collection date and soil but not to site. Average yields shown in table 1 indicate a tendency toward a large linear decrease as the season progressed.

The phosphorus content was significantly greater on the C soil, especially on good sites. The difference was significantly greater between C and B soils than between B and A soils. Phosphorus was higher on good sites at early and late dates of collection, but higher on poor sites at the intermediate date. Poor sites were higher in phosphorus on A soil but were lower in phosphorus on C soil. This soil-site interaction was highly significant.

CALCIUM

There was no significant difference in calcium content of the plant on different soil types (table 1). A highly significant increase was found in calcium at the middle date and this was followed by a decline, no significant difference existing between the early season and the late season analysis.

Plants from good sites tended to have slightly less calcium (1.43 percent) than those from poor sites (1.52 percent). However, the difference was not quite significant.

MAGNESIUM

Magnesium showed no significant relation to soil (table 1). There was a highly significant tendency toward decrease in average magnesium content as the season progressed. This was exhibited much more strongly in plants from good sites than in those from poor.

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Plants from poor sites contained a highly significantly greater quantity of magnesium, averaging 0.335 percent as compared to 0.290 in plants from good sites.

COMPARISON OF FIELD ERROR AND ANALYSIS ERROR

In order to place field errors on a comparable basis the variance of field error of each constituent was divided by the mean of the percent of that constituent in all samples. The same treatment was followed for analysis error. The results are shown in table 2.

From these values it is evident that a generally higher variability occurs between duplicate collection samples than between duplicate chemical analyses. The chemical analysis is most variable in crude fat, nitrogen-free extract, and crude fiber in the order named. This might be considered as an index to the accuracy of analytical methods used. The field-collection duplicates varied greatly in crude fiber, nitrogen-free extract, and crude fat in the order named.

Only in the case of crude fat did the variation within chemical-analysis duplicates exceed the variation between collection duplicates. This would indicate a refinement of analytical method insufficient to differentiate between collection duplicates. This same situation is approached in the case of nitrogen-free extract.

The greater variation shown in some constituents than in others between duplicate collections would indicate that these constituents vary more in individual plants, though it must be kept in mind that chemical-analysis error is carried over to field-collection error. For example, the high field error in the case of crude fat might be accounted for entirely by chemical error.

TABLE 2.—*Field- and laboratory-error comparison (variance divided by general mean) and ratio of laboratory error to field error by constituents of snowberry plants; based on data in table 1*

Constituent	Field error	Laboratory error	Ratio of laboratory error to field error	Constituent	Field error	Laboratory error	Ratio of laboratory error to field error
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
Protein	1.77	0.09	5.84	Total ash	4.57	.02	.44
Nitrogen-free extract	8.06	2.62	32.50	Phosphorus	.42	.004	.95
Crude fiber	18.08	.72	3.98	Calcium	1.89	.00	.00
Crude fat	6.90	10.93	158.40	Magnesium	2.75	.06	2.18

NEED OF DUPLICATE SAMPLING

Estimated standard errors were determined according to the method suggested by Bartlett and Greenhill (*2*). Table 3 shows these estimated errors for each constituent had field and laboratory duplication of various combinations been used. Mean square values derived from table 1 are shown in table 4.

With the exception of crude fat, there is little increase in accuracy gained by duplicate chemical analyses. As Bartlett and Greenhill have stated, "the chief justification of duplicate determinations * * * is that they serve as a check against the possible return of an erroneous figure." In the case of crude fat, however, there is apparent a large variation in chemical analyses, and duplication materially reduces the standard error.

Field-collection duplication generally does not give a significantly increased accuracy of data. Duplication of the field sampling, however, does appear to be more important than duplication of laboratory analyses. In the case of magnesium, duplicate collection results in a materially decreased standard error but in no other instance does the increased accuracy appear sufficient to justify the labor and cost involved. These conclusions do not, of course, ignore the importance of duplication as a means of checking errors.

TABLE 3.—*Estimated standard errors for each constituent of snowberry plants expressed as percentage of the general mean for various combinations of field and laboratory duplication; based on data in table 1*

Field samples	Laboratory samples	Protein	Nitrogen-free extract	Crude fiber	Crude fat [1]	Total ash	Phosphorus	Calcium	Magnesium
Number	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	1	22.84	5.84	24.89	9.00	13.24	32.37	10.96	21.04
1	2	22.83	5.60	24.85	5.90	13.24	32.37	10.96	21.04
2	1	22.74	5.59	24.43	9.08	12.54	31.68	9.37	14.60
2	2	22.73	5.33	24.40	6.58	12.54	31.68	9.37	14.60

[1] The larger standard error obtained for crude fat with 2 field samples probably is not significant and it means, simply, that the values obtained from 2 samples are no more reliable than those obtained from 1 sample.

TABLE 4.—*Mean square values for each constituent of snowberry plants by comparisons; based on data in table 1*

Comparison	Degrees of freedom	Protein, mean square	Nitrogen-free extract, mean square	Crude fiber, mean square	Crude fat, mean square	Ash, mean square	Phosphorus, mean square	Calcium, mean square	Magnesium, mean square
Month linear	1	260.634	292.349	1,305.836	1.394	27.271	0.129	0.000	0.062
Month quadratic	1	.566	40.768	125.142	.887	7.362	.010	.579	.014
Soil linear	1	.364	.011	7.192	3.752	2.750	.063	.052	.003
Soil quadratic	1	2.641	1.005	.506	.405	1.861	.090	.103	.000
Site	1	8.168	21.725	12.194	2.634	.467	.015	.124	.037
Month×soil	4	.980	6.713	9.103	.383	.091	.002	.043	.003
Month×site	2	2.125	1.765	1.192	.894	.429	.001	.008	.004
Soil×site	2	.956	1.387	.297	.751	.091	.031	.067	.001
Month×soil×site	4	.488	2.205	2.435	1.346	.337	.001	.030	.001
Σ Interaction	12	1.003	3.498	4.094	.850	.230	.006	.037	.002
Field error	18	.159	3.755	3.483	.818	.288	.001	.028	.009
Laboratory error	36	.008	1.218	.139	1.294	.001	.000	.000	.000

CONCLUSIONS

From these studies it is concluded that in collecting plants for complete chemical analysis attention must be given to soil type, site, and date of collection. The importance of these variables, however, is dependent upon the individual constituent being analyzed. In collections to determine seasonal variation it is essential to sample within the same site and soil type or so to composite the collection as to make it representative of these two variables. In complete chemical analysis the complicating interactions that occur between these variables make it impossible to forecast chemical response to any one factor unless all others are made constant or made a part of the analysis through appropriate experimental design.

For these reasons it is suggested that in sampling highly variable range floras for chemical analysis, seasonal collections be made from regularly established stations. These stations should be so located as to be representative of the soil types and sites that exist within the range. This can be done by making the number of stations within each soil type and site numerically proportionate to the area occupied by that particular soil type and site in the entire range. The sample should, of course, be equally composited from each station.

SUMMARY

Chemical response was studied in *Symphoricarpos rotundifolius* near Logan, Utah, to variation in site, date of collection, and soil type.

Date of collection was by far the most influential factor, late season resulting in a highly significant decrease in protein, nitrogen-free extract, total ash, phosphorus, and magnesium; and a highly significant quadratic influence upon calcium. Only crude fat failed to respond to season.

Soil type had a highly significant influence upon total ash and phosphorus content and a pronounced though insignificant effect upon protein content.

Site had a significant influence upon protein and a highly significant influence upon magnesium, protein being higher and magnesium lower on good sites. Nitrogen-free extract was also significantly higher on poor sites.

Complex interactions are common between date of collection, site, and soil, and for this reason attention must be given to all before broad conclusions are drawn regarding the influence of any.

Large error or variation was apparent in the analytical procedure for crude fat, and the field variability was especially high in crude fiber, nitrogen-free extract, and crude fat.

For the determination of most components duplicate collection and duplicate analysis do not increase the accuracy commensurate with the extra expense incurred, and hence can be justified only as a means of checking errors.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1930. official and tentative methods of analysis . . . Compiled by the committee on editing methods of analysis. Ed. 3, 593 pp., illus. Washington, D. C.

(2) Bartlett, M. S., and Greenhill, A. W.
1936. the relative importance of plot variation and of field and laboratory sampling errors in small plot pasture productivity experiments. Jour. Agr. Sci. [England] 26: [258]–262.

(3) Daniel, Harley A.
1934. the calcium, phosphorus, and nitrogen content of grasses and legumes and the relations of these elements in the plant. Amer. Soc. Agron. Jour. 26: 496–503.

(4) ——— and Harper, Horace J.
1935. the relation between effective rainfall and total calcium and phosphorus in alfalfa and prairie hay. Amer. Soc. Agron. Jour. 27: 644–652, illus.

(5) Fagan, T. W., and Milton, W. E. J.
1931. the chemical composition of eleven species and strains of grasses at different stages of maturity. Welsh Jour. Agr. 7: 246–255

(6) Gordon, Aaron, and Sampson, Arthur W.
1939. composition of common california foothill plants as a factor in range management. Calif. Agr. Expt. Sta. Bul. 627, 95 pp., illus.
(7) Greaves, J. E.
1938. the correlation among various constituents of forage plants. Amer. Soc. Agron. Jour. 30: 754–759.
(8) Holtz, Henry F.
1930. effect of calcium and phosphorus content of various soil series in western washington upon the calcium and phosphorus composition of oats, red clover, and white clover. Wash. Agr. Expt. Sta. Bul. 243, 45 pp.
(9) Hopper, T. H., and Nesbitt, L. L.
1930. the chemical composition of some north dakota pasture and hay grasses. N. Dak. Agr. Expt. Sta. Bul. 236, 38 pp., illus.
(10) McCreary, O. C.
1931. wyoming forage plants and their chemical composition—studies no. 9. Wyo. Agr. Expt. Sta. Bul. 184, 23 pp., illus.
(11) Orr, J. B.
1929. minerals in pastures & their relation to animal nutrition. 150 pp., illus. London.
(12) Snedecor, George W.
1938. statistical methods applied to experiments in agriculture and biology. Rev. ed., 388 pp., illus. Ames, Iowa.
(13) Stanley, E. B., and Hodgson, C. W.
1938. seasonal changes in the chemical composition of some important arizona range grasses. Ariz. Agr. Expt. Sta. Tech. Bul. 73: 451–466, illus.
(14) Truninger, E., and Grünigen, F. V.
1935. ueber den mineralstoffgehalt einiger unserer wichtigsten wiesenpflanzen mit besonderer berücksichtigung der physiologischen bedeutung des kalis im wiesenfutter. Landw. Jahrb. der Schweiz 49: [101]–127, illus.
(15) Watkins, James M.
1940. the growth habits and chemical composition of bromegrass, bromus inermis leyss, as affected by different environmental conditions. Amer. Soc. Agr. Jour. 32: 527–538.

AN INVERSION, A RECIPROCAL TRANSLOCATION, TRISOMICS, AND TETRAPLOIDS IN BARLEY [1]

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INTRODUCTION

The objectives of the investigation reported herein were (1) to explore the possibilities of barley (*Hordeum vulgare* L.) as a subject and source of problems for cytological study and (2) to make a survey of some mutants, varieties, and hybrids of barley in order to determine whether there may be any cytological basis for observed peculiarities, such as dwarfness or sterility, of certain plants. Except in a few instances, the observations here reported were made during June 1940 at Aberdeen, Idaho.

REVIEW OF LITERATURE

Though barley has been used rather extensively in genetic studies, few cytological observations have been reported. The first observers were interested in the number of chromosomes and their morphology in the various species. A review of the contributions of most of the early authors may be found in Ghimpu (*4*) [3] and Lewitsky (*10*).

Cultivated barleys, and some noncultivated species, have 14 somatic chromosomes. Wild species with 14, 28, and 42 chromosomes were reported by Griffee (*5*), Ghimpu (*4*), and others. The chromosomes are long and slender, with median or submedian centromeres. Emme (*3*) was the first to report satellites in barley. Lewitsky (*10*) reported that there were regularly 2 pairs of chromosomes with satellites in the species having 14 chromosomes. One pair of satellites was larger than the other. These satellites are presumably different from the large appendage observed by Hasegawa (*6*) in 9 species from *Aegilops*, *Triticum*, *Secale*, and *Hordeum*. If that is the case, there are 3 pairs of chromosomes with distinguishable appendages.

Johansen (*7*) examined root tips from a commercial lot of seed of *Hordeum vulgare* and found a high frequency (possibly 10 percent) of haploids. He observed two appendages (with inconclusive evidence of two more) in diploid and two in haploid root tips. The haploids were discarded before their constitution was known, so a study of meiosis could not be made in them. Tometorp (*23*) studied meiosis in pollen mother cells of a haploid of *H. distichum*, however, and found occasional bivalents. Usually the chromosomes were dis-

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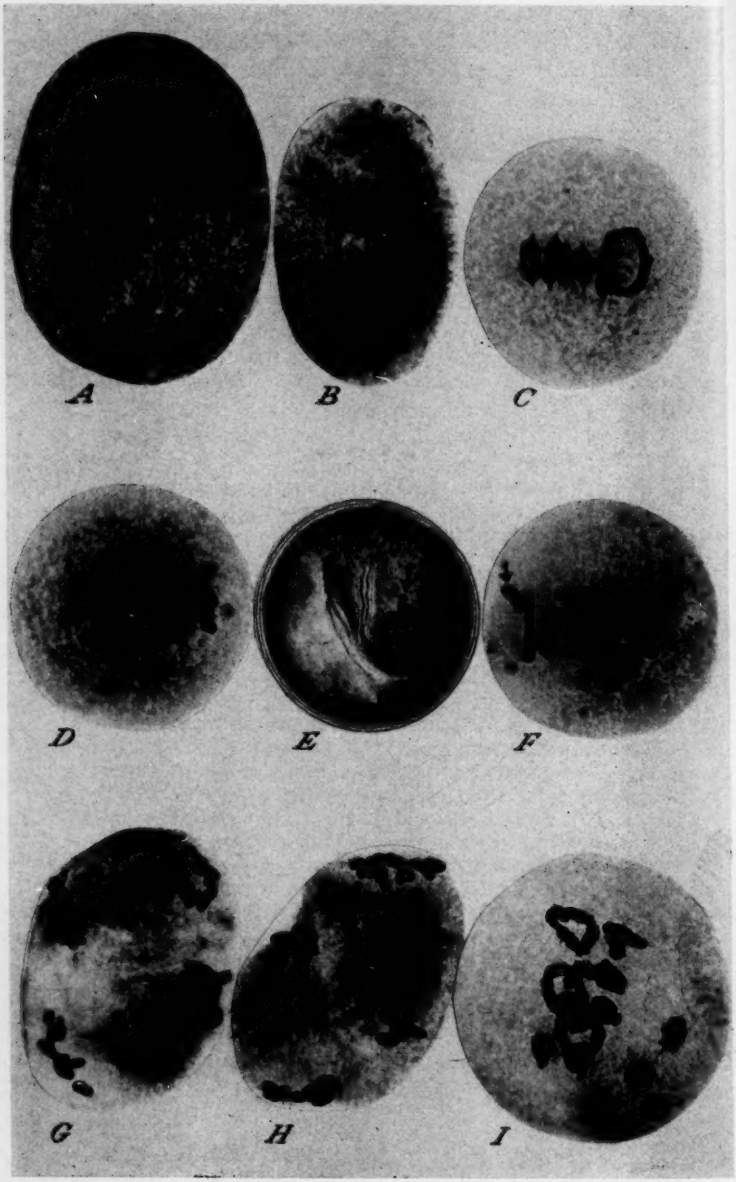
[3] Italic numbers in parentheses refer to Literature Cited, p. 749.



(See Explanatory Legend on opposite page)

tributed at random in the first division and the chromatids did not ordinarily separate until the second division. Microspores were observed with 7, 8, and 13 or 14 chromosomes at the first mitotic division.

Ekstrand (*2*) reported observations on the meiotic process in a partially asynaptic strain of barley, and Swenson (*22*) described meiosis in normal plants.

Peto (*13*) examined diploid and tetraploid florets on the same spike for chromosome pairing and chiasma frequency. He found an average of 3.58 quadrivalents (range 1 to 7) in 4*n* pollen mother cells. One of two megaspore mother cells had 7 quadrivalents; the other had 4. In the tetraploid pollen mother cells there were 1.87 times as many chiasmata as in the diploid.

Müntzing, Tometorp, and Mundt-Petersen (*12*) and Karpechenko (*8*), with heat, and Dorsey (*1*), with colchicine, obtained tetraploid barley plants. All found plants with 1, 2, or 3 more than 28 somatic chromosomes in the progeny of tetraploid parents. Müntzing reported 4, and Karpechenko 1, offspring with only 27 chromosomes.

Kattermann (*9*) was the first to report a trisomic plant in barley. He observed mitotic and meiotic divisions in a trisomic plant of *Hordeum distichum*. The chromosome involved was not one with a satellite.

MATERIAL AND METHODS

The plants studied were mainly from varieties and strains of *Hordeum vulgare* that were being used in the barley breeding project of the Division of Cereal Crops and Diseases. An adapted acetocarmine smear technique was used in making all slides for cytological observations. Carnoy's fluid was used as a fixative. Heads were fixed for 1 to several days; root tips for a week or longer. A more complete description of the root-tip smear procedure has been published by Smith (*18*). Some smears were made permanent by an improved method (employing tertiary butyl alcohol instead of xylene) suggested by Sears (*17*). Photomicrographs were taken with a Leitz Makam camera.

A, A bridge with a fragment beside it; first anaphase in a pollen mother cell. *B*, A bridge and fragment at second telophase in one cell of a dyad. The fragment is indicated near the pole. *C*, A ring of four chromosomes (open) at first metaphase in a pollen mother cell of a plant heterozygous for a reciprocal translocation. *D*, Seven pairs at first metaphase from the same slide as *C*. Two pairs are attached at only one end. *E*, First division in a microspore, showing satellites on two chromosomes. *F*, First meiotic metaphase in a pollen mother cell of trisomic plant 202–1; trisome indicated by arrow. *G*, Second meiotic anaphase in a pollen mother cell of trisomic plant 202–2. A lagging chromatid is in each daughter cell. The chromatids of the extra chromosome presumably separated in the first division. *H*, A chromatid in which "misdivision" has occurred; second meiotic anaphase in trisomic plant 202–2. *I*, Diakinesis in a tetraploid "Big Boy," barley. There are three open rings of four, a zigzag group of four (top, right), a chain of four (center, right), and four bivalents. Note the two nucleoli. (Photomicrographs *A* and *B* were taken at a magnification of 1,050 diameters, the others at 850. All were reduced about 1/10 in reproduction.)

AN INVERSION

In attempting to determine the cause of the low seed production in a "Sterile Flowers" stock (a selection from *Hordeum deficiens nudideficiens*, C. I.[4] No. 2229), two of five plants examined in one progeny were found to have seven pairs of chromosomes at first metaphase and bridges and fragments at first and second anaphase or telophase in pollen mother cells (pl. 1, *A* and *B*). Other "Sterile Flowers" plants did not have the bridges and fragments, so presumably there is some other cause for the sterility in "Sterile Flowers" plants. Table 1 summarizes the observations on bridges and fragments at the first division in the two plants. In plant 219–2, 24 percent of the cells had either a bridge or both a bridge and a fragment, and in plant 219–4 the value was 14 percent. A larger proportion of the cells of plant 219–4 were without visible fragments.

At second anaphase and early telophase in plant 219–2, 4 quartets had a bridge and fragment; 16 had a bridge but no visible fragment; and 72 had neither bridge nor fragment. Thus about 20 percent of the quartets had a bridge. The fragment was not necessarily located near the bridge. The fragment presumably was formed in the prophase of the first division, though it may not have become free until the second.

TABLE 1.—*Data on bridges and fragments at the first meiotic division in pollen mother cells of plants with an inversion*

Plant	Cells with a bridge		Cells without a bridge		Total cells
	With a fragment	Without a fragment	With a fragment	Without a fragment	
	Number	*Number*	*Number*	*Number*	*Number*
219–2	18	5	5	67	95
219–4	8	21	9	170	208

The bridges and fragments in the two plants may be accounted for by assuming single cross-overs in an inverted segment, as depicted in figure 1, though, as shown by Richardson (*15*), a duplication can also give bridges and fragments in plants with bivalents only. As the fragment is small the inversion must be near the end of the chromosome. The percentage of bridges, however, might lead one to expect a larger fragment. The explanation may be a high frequency of crossing over localized in the short inverted segment. The bridges at second anaphase and telophase fit the interpretation of single cross-overs in the inverted region, accompanied by simultaneous cross-overs in the proximal uninverted portion of the arm. Explanations for the absence of a fragment in a cell with a bridge at the first division have been considered by McClintock (*11*), Swanson (*21*), and others.

Plant 219–2 (a 2-rowed type) had a seed in all but 1 of 45 primary florets, so female fertility was not much reduced.

McClintock[5] has also observed that inversions (and other aberrations which give rise to bridge configurations at meiosis) do not

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases.
[5] MCCLINTOCK, B. Unpublished observations.

produce female sterility proportionate to the number of pollen mother cells with bridges.

Sturtevant and Beadle (*19*) and others have observed that single cross-overs in heterozygous inversions in *Drosophila melanogaster* do not result in inviable zygotes. Sturtevant and Beadle suggested that the chromosomes were oriented in such a manner that only non-cross-over chromatids get into the nuclei that become the egg nuclei. The same sort of behavior may account for the lack of sterility in the barley plant.

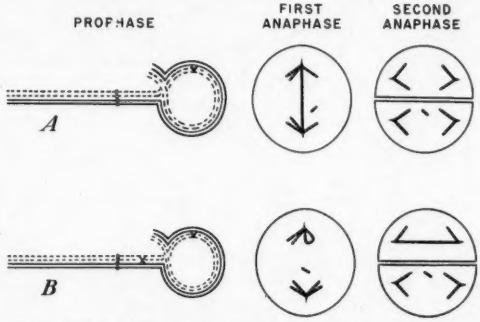


FIGURE 1.—A diagram illustrating some modes of origin of bridges and fragments from crossing over in a bivalent heterozygous for an inversion. *A*, A single cross-over in an inverted segment. *B*, A single cross-over in an inverted segment and a simultaneous cross-over in a proximal uninverted segment producing a three-strand double cross-over. Cross-overs are represented by "x" and the centromeres by circles. For more complete diagrams of crossing over in inversions, see McClintock (*11*), Richardson (*15*), and Sax (*16*).

A RECIPROCAL TRANSLOCATION

In 1938 the writer received from G. A. Wiebe [6] seed of plants, some of which were supposed to be heterozygous for a gene causing "Contabescent Anther," a mutant characterized by nearly complete male and female sterility. This mutant was selected from C. I. No. 3845, a variety obtained from Kashmir, India. The sterility is associated with a meiotic irregularity as yet undescribed. In an examination of this stock for the meiotic disturbance, the reciprocal translocation described in this section and the three trisomic plants reported in the next section were found. No cause for the coincidence of these three different irregularities in the same stock was observed. Several normal plants were regular at meiosis.

The translocation apparently involves small segments of each of two nonhomologous chromosomes. Often the configuration at first metaphase is a chain of four instead of a ring, and not infrequently seven pairs are formed (pl. 1, *C* and *D*, and table 2).

More pollen mother cells had zigzag (disjunctional) than open (nondisjunctional) configurations of the chromosomes in the ring or chain (table 2). Plant 201–1 was the parent of 207–1, and the behavior of the ring in the two plants was similar. The behavior of

[6] Agronomist, Division of Cereal Crops and Diseases.

the ring in the two sib plants of 203, whose grandparent was the parent of 201, differed from that of the ring in 201 and 207 in that fewer rings and chains were formed and more cells had seven pairs. It is possible that another interchange is involved, though the close relationship between 201, 207, and 203 makes this unlikely. Also interchanges involving such small segments that chains and even pairs are formed have not been common in other organisms. Except for the closed rings in the two plants of 203, these plants might be suspected of having received deficiency-duplication gametes formed by nondisjunctional segregations of the ring observed in 201 and 207.

TABLE 2.—*Configurations of chromosomes at first meiotic metaphase in pollen mother cells of plants heterozygous for a reciprocal translocation*[1]

Plant	Ring of 4			Chain of 4			7 pairs	Unclassified	Total cells
	Zigzag	Open	Unclassified	Zigzag	Open	Unclassified			
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
201–1	83	48	2	39	19	2	12	16	221
207–1	52	29	1	99	37	7	54	39	318
203–1	2	2	0	34	23	0	119	48	228
203–2	8	12	0	32	21	0	31	122	226

[1] See text for relationship of plants.

No comparison of the seed set of normal and ring-forming sib plants has been made as yet, but it is clear from table 3 that plants with the interchange are not "semisterile" in seed set. The first two plants in table 3 were ring-forming plants grown in the greenhouse at Columbia, Mo. The third was grown in the field at Aberdeen, Idaho. The last two plants were normal field plants which were not sibs of the ring-forming plant but do give some information on fertility of plants of similar type. There is a close agreement in the fertility of the three ring-forming plants (65.6, 69.0, and 64.8 percent) and a similar agreement between the two seven-pair plants (92.4 and 93.0 percent). The fertility of the ring-forming plants also approximates the proportion of the pollen mother cells of the first two plants of table 2, in which the chromosomes were oriented disjunctionally.

TABLE 3.—*Fertility of ring-forming and normal plants*

Plant	Configuration	Florets	Seed	Fertility
		Number	*Number*	*Percent*
347–1	5 pairs + R_4	96	63	65.6
347–2	do	129	89	69.0
207–2	do	270	175	64.8
216–2	7 pairs	198	183	92.4
217–1	do	213	198	93.0

TRISOMICS

Three trisomic plants were found in the same stock that had the reciprocal translocation and the mutant "Contabescent Anther." Two occurred among 182 offspring of 1 plant. The third was found among about 20 offspring from another plant. The populations were so uniform that the 3 trisomic plants, which were smaller than the others,

were suspected of being haploid or otherwise abnormal. It is possible that other trisomics were present but did not affect the appearance of the plants sufficiently to attract attention. The 3 plants were about two-thirds the height of normal plants, were vigorous, and produced several tillers. There was no obvious morphological characteristic to distinguish them from one another or from normal plants. Careful observations on more individuals may reveal identifiable peculiarities.

There is probably no causal relation between the translocation previously described and the trisomes. If there had been a 3 : 1 distribution of the chromosomes at meiosis in a ring-forming plant, the offspring receiving the extra chromosome would have a chain of five chromosomes in some pollen mother cells. No more complex association than a trivalent was observed in the trisomic plants, but since the translocation apparently involves quite small segments it is difficult to exclude the possibility that chains of five do occur.

Cytologically there was no clue as to whether the same chromosome was involved in the three trisomic plants. Cytological observations were limited, however, to meiotic stages, where chromosome morphology is not very well expressed. Further observations on root tips or microspores should make it possible to determine whether either of the satellited chromosomes as shown in plate 1, *E*, and by Lewitsky (*10*) is present in triplicate.

In some pollen mother cells the chromatids of the extra chromosome separated in the first division. These presumably accounted for the lagging chromosomes at the second division and for most of the micronuclei in the quartets.

Photomicrographs (pl. 1, *F* and *G*) show typical configurations of meiosis in the trisomic plants. Table 4 summarizes the observations on the extra chromosome in two of the plants. A considerable proportion of the quartets had no micronucleus and approximately one-third of the spores probably had eight chromosomes (calculated from table 4). There was no observed cause for the considerable number of quartets in plant 202–1 with more than two micronuclei. Four quartets in this plant had five micronuclei each; others had three or four.

The chromosomes of a trisome in trisomic plants are known to tend to "misdivide"; i. e., to divide transversely instead of longitudinally across the centromere. As a result of this "misdivision" half chromosomes are formed with terminal centromeres.[7] This sort of behavior was observed in several pollen mother cells in the trisomic barley plant 202–2. Plate 1, *H*, shows a chromatid after a "misdivision." The irregularity in this case probably occurred at the second anaphase, though in another cell at second metaphase two fragments were already present so the "misdivision" in this case presumably had occurred in the first division. From results reported in other organisms, half chromosomes with terminal centromeres and secondary trisomes might be expected if sufficiently large progenies from the trisomic plants were examined.

Trisomic offspring have been obtained from two of the three original plants (202–2 and 207–3).

[7] See Rhoades (*14*) for a review of the literature relating to this phenomenon.

TABLE 4.— *Observations on meiosis in trisomic plants*

Plant	First metaphase		First anaphase		First telophase			Second anaphase			Second telophase			
	Cells with trivalents	Cells with univalents	Cells with chromatids separating	Cells with chromatids not separating	Cells with indicated number of micronuclei			Cells with indicated number of lagging chromosomes			Quartets with indicated number of micronuclei			
					0	1	2	0	1	2	0	1	2	3 or more
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
202–1	4	43			41	4	0				95	40	62	20
202–2	12	25	14	14	106	12	1	5	2	2	33	13	10	0

TETRAPLOIDS

Three tetraploid stocks were available for study, but because of the lack of time and the abundance of other material only limited observations were made on them. One stock, "Big Boy," was found in 1936 by H. V. Harlan [8] in a selection from the cross Trebi × Club Mariout; another was a tetraploid strain of Opal B [9]; the third stock was a tetraploid line of the variety Everest, obtained by Harland Stevens [10] from a treatment of germinating seeds with colchicine.

There was no conspicuous difference in the meiotic behavior of 4*n* plants from the three sources. Quadrivalents were the rule (pl. 1, *I*). Univalents and trivalents were fairly common, but micronuclei in the quartets were not numerous.

Each of the three lines had occasional small plants; some were extreme dwarfs, forming rosettelike clumps. Some of the less abnormal plants were found to have 26 or 27 chromosomes, but no count was obtained on the more extreme dwarfs.

Stevens had planted seeds from a few small plants of the 4*n* Everest stock, and in turn the small plants in these progenies were examined. One of the smaller plants had 29 chromosomes, another 29 or 30, and a third had 28. This last plant possibly had an unbalanced constitution, with more than 4 homologous chromosomes in some cases and fewer than 4 in others.

Six plants that grew from attempted crosses of diploid pollinated by tetraploid had 14 chromosomes at meiosis. One plant from the attempted cross of tetraploid by diploid had 28 chromosomes in a root tip.

Table 5 presents data on the fertility of the three tetraploid strains. Although the data on corresponding diploid lines are not available, it

TABLE 5.—*Fertility of tetraploid barleys*

Tetraploid strain	Location where grown	Year	Florets	Seed	Fertility
			Number	*Number*	*Percent*
Big Boy	Aberdeen, Idaho	1936	193	123	63.7
Do	Madison, Wis.	1940	153	70	45.8
Opal B	do	1940	64	26	40.6
Do	Sacaton, Ariz.	1940	120	42	35.0
Everest	Aberdeen, Idaho	1940	357	160	44.8

[8] Principal agronomist, Division of Cereal Crops and Diseases.
[9] Obtained from A. Müntzing, of Svalof, Sweden.
[10] Assistant agronomist, Division of Cereal Crops and Diseases.

can be safely assumed from this table that fertility in tetraploids is considerably lower than in the diploids.

OBSERVATIONS ON MISCELLANEOUS MUTANTS AND HYBRIDS IN BARLEY

Seven mutants (in addition to "Contabescent Anther") were examined for meiotic irregularities. No abnormality was observed at the stages examined, which in almost every case included first metaphase and anaphase, but the observations were of necessity limited. The mutants included: "Curly Awns," one plant; "Sterile Flowers," seven plants; "Sterile Flowers, Smooth Stigmas," one plant; "All Wrong" (a dwarf), three plants; "Mary's Dwarf," one plant; "Multan" (a variety), two plants; "Uniculm," two plants; Suneson's "Male Sterile" (*20*), three plants.

In a search for trisomics, triploids, and haploids, offtype plants were sampled among the large number of varietal hybrids in the breeding project at Aberdeen. No chromosomal aberrations were found though 29 peculiar plants were examined.

SUMMARY

About 20 percent of the pollen mother cells in two plants of one progeny of barley had a bridge or fragment or both at the first division. These configurations are interpreted as due to crossing over in a heterozygous inversion.

A reciprocal translocation is described. Limited observations indicate that in about 60 percent of the pollen mother cells, segregations of the chromosomes were disjunctional. Fertility was about 65-percent perfect in three ring-forming plants.

Three trisomic plants were found. Observations on meiosis in two of the plants are recorded.

Observations are reported on meiosis, fertility, and chromosome number in three strains of tetraploid barley.

Examination of a number of mutants and varietal hybrids revealed a meiotic disturbance as characteristic of only one mutant.

LITERATURE CITED

(1) DORSEY, E.
1939. CHROMOSOME DOUBLING IN THE CEREALS. Jour. Hered. 30: 393–395, illus.

(2) EKSTRAND, H.
1932. EIN FALL VON ERBLICHER ASYNDESE BEI HORDEUM. Svensk Bot. Tidskr. 26: [292]–302, illus.

(3) EMME, H.
1925. BEITRÄGE ZUR CYTOLOGIE DER GERSTEN. I. KARYOTYPEN DER GERSTEN. Ztschr. f. Induktive Abstam. u. Vererbungslehre 37: 229–236, illus.

(4) GHIMPU, VICTOR.
1930. RECHERCHES CYTOLOGIQUES SUR LES GENRES: HORDEUM, ACACIA, MEDICAGO, VITIS, ET QUERCUS. Arch. d'Anat. Micros. 26: [135]–234, illus.

(5) GRIFFEE, FRED.
1927. CHROMOSOME NUMBER IN SPECIES OF HORDEUM. Minn. Univ. Studies Biol. Sci. 6: 319–331, illus.

(6) HASEGAWA, NOBUMI.
1934. CHROMOSOME STUDIES ON THE POLLEN NUCLEI OF SOME CEREALS. Jap. Jour. Genet. 10: [84]–88, illus. [In Japanese. English summary, pp. 87–88.]
(7) JOHANSEN, DONALD A.
1934. HAPLOIDS IN HORDEUM VULGARE. Natl. Acad. Sci. Proc. 20: 98–100, illus.
(8) KARPECHENKO, G. D.
1938. TETRAPLOID BARLEYS OBTAINED BY HIGH TEMPERATURE TREATMENT. Biol. Zhur. 7: 287–294, illus. [In Russian. English summary, pp. 291–294.]
(9) KATTERMANN, G.
1939. STERILITÄTSSTUDIEN BEI HORDEUM DISTICHUM. Ztschr. f. Induktive Abstam. u. Vererbungslehre 77: 53–103.
(10) LEWITSKY, G. A.
1931. THE MORPHOLOGY OF THE CHROMOSOMES. HISTORY. METHODS. FACTS. THEORY. Trudy Prikl. Bot., Genet., i Selek. (Bul. Appl. Bot., Genet. and Plant Breeding) 27: [19]–173, illus. [In Russian. English translation, pp. [103]–169.]
(11) MCCLINTOCK, BARBARA.
1938. THE FUSION OF BROKEN ENDS OF SISTER HALF-CHROMATIDS FOLLOWING CHROMATID BREAKAGE AT MEIOTIC ANAPHASES. Mo. Agr. Expt. Sta. Res. Bul. 290, 48 pp., illus.
(12) MÜNTZING, A., TOMETORP, G., and MUNDT-PETERSEN, K.
1937. TETRAPLOID BARLEY PRODUCED BY HEAT TREATMENT. Hereditas 22: [401]–406, illus.
(13) PETO, F. H.
1936. HEAT INDUCED TETRAPLOIDY IN BARLEY. Canad. Jour. Res., Sect. C, Bot. Sci. 14: 445–447, illus.
(14) RHOADES, M. M.
1940. STUDIES OF A TELOCENTRIC CHROMOSOME IN MAIZE WITH REFERENCE TO THE STABILITY OF ITS CENTROMERE. Genetics 25: 483–520, illus.
(15) RICHARDSON, M. MARGARET.
1936. STRUCTURAL HYBRIDITY IN LILIUM MARTAGON ALBUM × L. HANSONII. Jour. Genet. 32: [411]–450, illus.
(16) SAX, KARL.
1937. CHROMOSOME INVERSIONS IN PAEONIA SUFFRUTICOSA. Cytologia Fujii Jubilee, pp. 108–114, illus.
(17) SEARS, E. R.
—— CHROMOSOME PAIRING AND FERTILITY IN HYBRIDS AND AMPHIDIPLOIDS IN THE TRITICINAE. Mo. Agr. Expt. Sta. Res. Bul. [in press.]
(18) SMITH, LUTHER.
1939. MUTANTS AND LINKAGE STUDIES IN TRITICUM MONOCOCCUM AND T. AEGILOPOIDES. Mo. Agr. Expt. Sta. Res. Bul. 298, 26 pp., illus.
(19) STURTEVANT, A. H., and BEADLE, G. W.
1936. THE RELATIONS OF INVERSIONS IN THE X CHROMOSOME OF DROSOPHILA MELANOGASTER TO CROSSING OVER AND DISJUNCTION. Genetics 21: 554–604, illus.
(20) SUNESON, C. A.
1940. A MALE STERILE CHARACTER IN BARLEY; A NEW TOOL FOR THE PLANT BREEDER. Jour. Hered. 31: 213–214.
(21) SWANSON, CARL R.
1940. THE DISTRIBUTION OF INVERSIONS IN TRADESCANTIA. Genetics 25: 438–465, illus.
(22) SWENSON, S. P.
1940. GENETIC AND CYTOLOGIC STUDIES OF A BRACHYTIC MUTATION IN BARLEY. Jour. Agr. Res. 60: 687–713, illus.
(23) TOMETORP, GÖSTA.
1939. CYTOLOGICAL STUDIES ON HAPLOID HORDEUM DISTICHUM. Hereditas 25: [241]–254, illus.

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